Rec'd PCT/PTO 02 APR 2001

FORM PTO-1390 (Modified) (REV 11-98) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: **205399US0XPCT**

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR): 09/787923

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/FR99/02329	**30 September 1999**	**02 October 1998**

TITLE OF INVENTION

CHEMICAL STRUCTURE HAVING AN AFFINITY FOR A PHOSPHOLIPID AND LABELLING COMPOUND, DIAGNOSE KIT, AND DRUG COMPRISING THIS STRUCTURE

APPLICANT(S) FOR DO/EO/US

Alain SANSON, et al.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ A copy of the International Search Report (PCT/ISA/210).
8. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
9. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
10. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
11. ☐ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☒ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).

Items 13 to 20 below concern document(s) or information included:

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☐ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☐ Certificate of Mailing by Express Mail
20. ☒ Other items or information:

Request for Consideration of Documents Cited in International Search Report
Notice of Priority
PCT/IB/304
Drawings (10 Sheets)
Amended Sheets(Pages 10, 11, 38, 39, 40, 41, 42, 43 and 44)

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
09/787923	PCT/FR99/02329	205399US0XPCT

21. The following fees are submitted:.

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :

		CALCULATIONS PTO USE ONLY
☐	Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2) paid to USPTO and International Search Report not prepared by the EPO or JPO	$1,000.00
☒	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but Internation Search Report prepared by the EPO or JPO	$860.00
☐	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO	$710.00
☐	International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4)	$690.00
☐	International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4)	$100.00

ENTER APPROPRIATE BASIC FEE AMOUNT =	$860.00	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☒ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).	$130.00	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	- 20 =	0	x	$18.00	$0.00
Independent claims	- 3 =	0	x	$80.00	$0.00
Multiple Dependent Claims **(check if applicable)**.			☐		$0.00

TOTAL OF ABOVE CALCULATIONS =	$990.00	
Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28) **(check if applicable)**. ☐	$0.00	
SUBTOTAL =	$990.00	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)). +	$0.00	
TOTAL NATIONAL FEE =	$990.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).** ☐	$0.00	
TOTAL FEES ENCLOSED =	$990.00	
Amount to be: refunded	$	
charged	$	

☒ A check in the amount of **$990.00** to cover the above fees is enclosed.

☐ Please charge my Deposit Account No. in the amount of to cover the above fees.
A duplicate copy of this sheet is enclosed.

☒ The Commissioner is hereby authorized to charge any fees which may be required, or credit any overpayment to Deposit Account No. **15-0030** A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

22850

Surinder Sachar
Registration No. 34,423

Surinder Sachar
SIGNATURE

Norman F. Oblon
NAME

24,618
REGISTRATION NUMBER

April 2 2001
DATE

#6

DOCKET NO.: 205399US0XPCT

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF: :

Alain SANSON et al : ATTN: BOX SEQUENCE

SERIAL NO: 09/787,923 :

FILED: April 2, 2001 :

FOR: CHEMICAL STRUCTURE HAVING:
AN AFFINITY FOR A PHOSPHOLIPID
AND LABELLING COMPOUND,
DIAGNOSE KIT, AND DRUG COMPRISING
THIS STRUCTURE

PRELIMINARY AMENDMENT

ASSISTANT COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231

SIR:

Responsive to the Official Correspondence dated July 16, 2001, and in accordance with the provisions of 35 U.S.C. §371, Applicants submit herewith amendments to the specification, a Sequence Listing, and a corresponding computer-readable Sequence Listing. Prior to examination on the merits, please amend the above-identified application as follows.

IN THE SPECIFICATION

Page 50 (Abstract), after the last line, beginning on a new page, please insert the attached Sequence Listing.

REMARKS

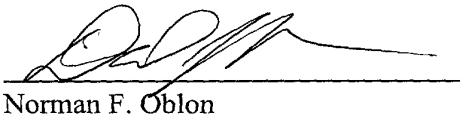
Claims 1-115 are active in the present application.

Applicants have now submitted a Sequence Listing and a corresponding computer-readable Sequence Listing. Contents of the paper copy of the Sequence Listing and the computer-readable Sequence Listing are identical. Support for all the sequences listed in the Sequence Listing can be found in the present application. No new matter is introduced by the submission of the Sequence Listing and the computer-readable Sequence Listing.

Applicants submit that the present application is now in condition for examination on the merits. Early notice to this effect is earnestly solicited.

Respectfully submitted,

OBLON, SPIVAK, McCLELLAND,
MAIER & NEUSTADT, P.C.



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22850

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205399US-0X PCT

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF: :

ALAIN SANSON ET AL : ATTN: APPLICATION DIVISION

SERIAL NO: 09/787,923 :

FILED: APRIL 2, 2001 : EXAMINER:

FOR: CHEMICAL STRUCTURE HAVING:
AN AFFINITY FOR A PHOSPHOLIPID
AND LABELLING COMPOUND, DIAGNOSE
KIT, AND DRUG COMPRISING THIS
STRUCTURE

PRELIMINARY AMENDMENT

ASSISTANT COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231

SIR:

Prior to examination on the merits, please amend the above-identified application as follows:

IN THE CLAIMS

Please amend the claims as follows:

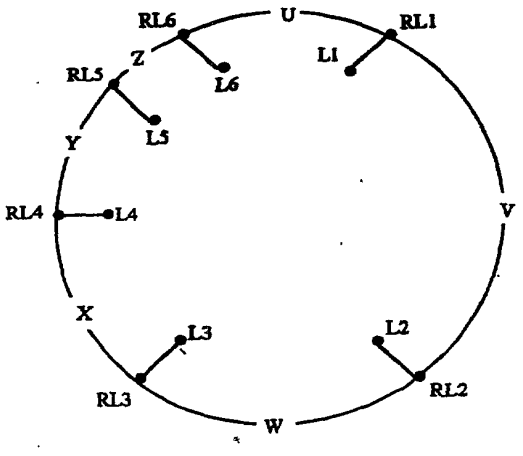
10. (Amended) The chemical structure according to Claim 1, wherein the platform is a portion of a domain of the annexin or of a modified domain of the annexin, comprising at least said residual ligands, RL1 to RL6, having said functions L1 to L6 for binding to the phospholipid respectively.

15. (Amended) The chemical structure according to Claim 13, wherein M is a peptide consisting of 33 natural or non-natural amino acids.

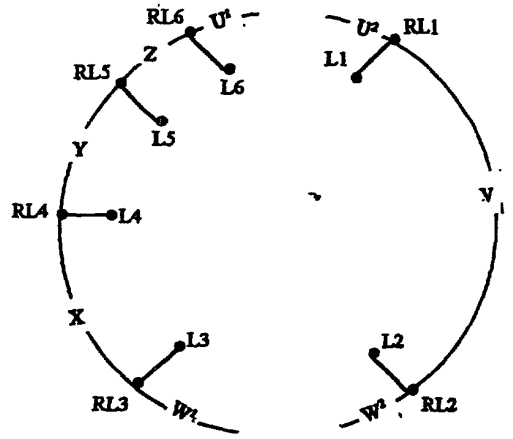
19. (Amended) The chemical structure according to Claim 13, further comprising a calcium site where the calcium ion is complexed by this site forms one of the ligands of the negatively charged phospholipid.

20. (Amended) The chemical structure according to Claim 1, said structures having an affinity for a phospholipid selected from a phosphatidylserine, a phosphatidylethanolamine, a phosphatidylinositol, a phosphatidic acid, and a cardiolipid.

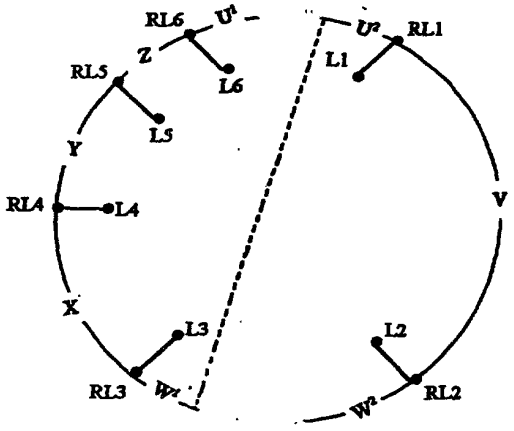
21. (Amended) A chemical assembly having an affinity for a phospholipid, characterized in that it comprises at least two identical or different chemical structures selected from the group consisting of A, B, C, D and E where A is a chemical structure with an affinity for a phospholipid, consisting of at least a chemical platform U, V, W, X, Y, Z including six residues RL1, RL2, RL3, RL4, RL5, RL6 supporting a set of chemical functions which may bind to said phospholipid, called, L1, L2, L3, L4, L5, L6 respectively, wherein these chemical functions L define the affinity of said structure for said phospholipid, said structure having one of the following constructions (I), (II) and (III):



(I)



(II)

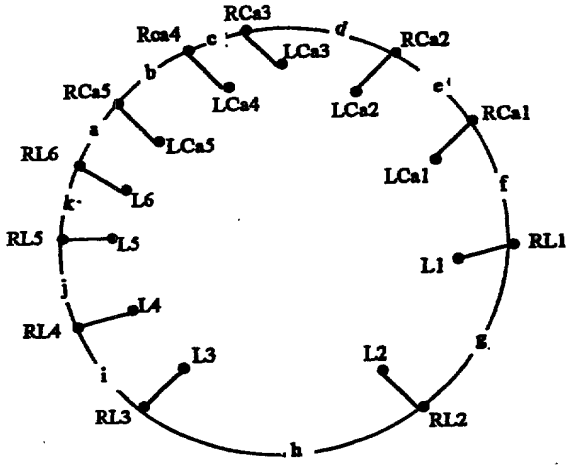


(III)

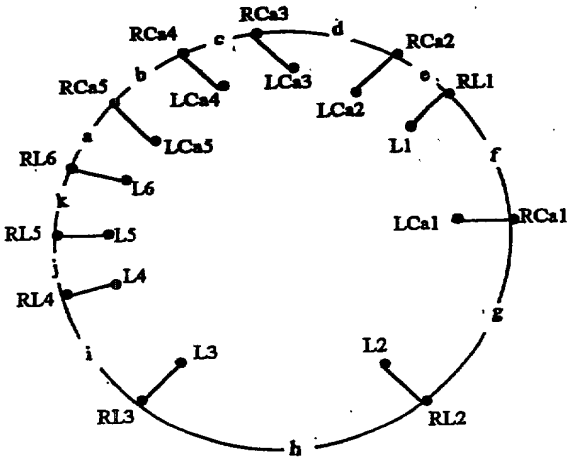
wherein U, U^1, U^2, V, W, W^1, W^2, X, Y, Z are independently a natural or non-natural amino-acid, a peptide consisting of natural or non-natural amino-acids, a carbon chain, or carbon cyclic group(s),

wherein RL1 to RL6 are selected from molecules having the binding chemical functions L1 to L6, respectively, wherein said chemical functions comprise either at least a positive charge, donor of a hydrogen bond, or at least a negative charge, acceptor of a hydrogen bond, and

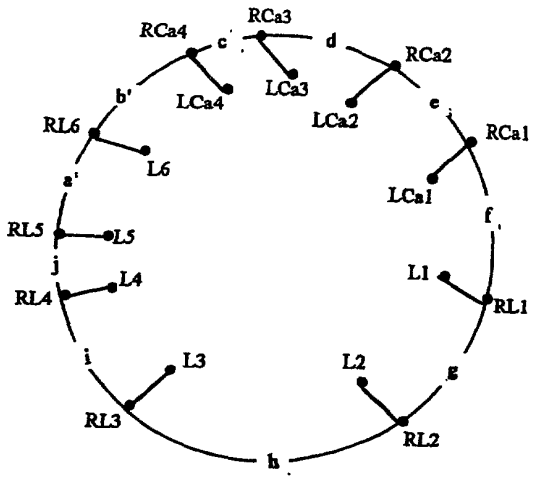
wherein U, U^1, U^2, V, W, W^1, W^2, X, Y, Z are such that RL6 and RL1 are distant from 0.65 to 0.95 nm, L6 and L1 are distant from 0.65 to 0.9 nm, RL1 and RL2 are distant from 0.45 to 0.65 nm, L1 and L2 are distant from 0.4 to 0.55 nm, RL2 and RL3 are distant from 0.5 to 1.05 nm, L2 and L3 are distant from 0.4 to 0.6 nm, RL3 and RL4 are distant from 0.5 to 0.8 nm, L3 and L4 are distant from 0.35 to 0.5 nm, RL4 and RL5 are distant from 0.45 to 0.75 nm, and L4 and L5 are distant from 0.4 to 0.55 nm, RL5 and RL6 are distant from 0.4 to 1.2 nm, L5 and L6 are distant from 0.4 to 0.6 nm, where B is a chemical structure with an affinity for a phospholipid, consisting of at least a chemical platform a, a', b, b', c, d, e, f, g, h, i, j, k, l including 11 residues, LR1, LR2, LR3, LR4, LR5, RL6, RCa1, RCa2, RCa3, RCa4 and RCa5 supporting a set of chemical functions which may bind to said phospholipid called L1, L2, L3, L4, L5, L6, respectively, and a set of chemical functions binding to a calcium atom called LCa1, LCa2, LCa3, LCa4, LCa5, respectively, wherein these chemical functions RL1 to RCa5 define the affinity of said structure for said phospholipid, said structure having one of the following constructions (IV), (V) and (VI):


RCa3
Rca4
c
d
RCa2
LCa3
b
RCa5
LCa4
LCa2
e
RCa1
a
LCa5
RL6
LCa1
f
k
L6
RL5
L5
RL1
L1
j
L4
RL4
g
L3
L2
i
RL3
RL2
h

(IV)


RCa3
RCa4
c
d
RCa2
b
LCa3
RCa5
LCa4
LCa2
e
RL1
a
LCa5
L1
RL6
f
k
L6
LCa1
RCa1
RL5
L5
j
L4
RL4
g
i
L3
L2
RL3
RL2
h

(V)



(VI)

wherein a, a', b, b', c, d, e, f, g, h, i, j, k, l, are independently a natural or non-natural amino acid, a peptide consisting of natural or non-natural amino acids, a carbon chain, or carbon cyclic group(s),

wherein RL1 to RL6 and RCa1 to RCa5 are selected from molecules having chemical binding functions L1 to L6 and LCa1 to LCa5, respectively, wherein said chemical functions L1 to L6 comprise either at least a positively charged donor of a hydrogen bond, or at least a negatively charged acceptor of a hydrogen bond, said chemical functions LCa1 to LCa5 comprising an oxygen atom, and

wherein a in the structures of construction (IV) and (V) is such that RL6 and RCa5 are distant from 0 to 0.35 nm and such that L6 and LCa5 are distant from 0 to 0.3 nm, b in the structures of construction (IV) and (V) is such that RCa5 and RCa4 are distant from 0 to 0.35 nm and such that LCa5 and LCa4 are distant from 0.2 to 0.3 nm, b' in the structure of construction (VI) is such that RL6 and RCa4 are distant from 0 to 0.35 nm and such that L6 and LCa4 are distant from 0 to 0.35 nm, c and d are such that RCa4 and RCa3 are distant from 0.5 to 0.9 nm, LCa4 and LCa3 are distant from 0.2 to 0.4 nm, RCa3 and RCa2 are

distant from 0.35 to 0.6 nm, and LCa3 and LCa2 are distant from 0.22 to 0.3 nm, e, f, g, in the structures of construction (IV), (V), (VI) are such that RL1 and RL2 are distant from 0.45 to 0.65 nm, RCa1 to RCa2 are distant from 0.4 to 0.55 nm, L1 and L2 are distant from 0.4 to 0.55 nm and LCa1 and LCa2 are distant from 0.3 to 0.4 nm, h, i, j and k are such that RL2 and RL3 are distant from 0.5 to 1.05 nm, L2 and L3 are distant from 0.4 to 0.6 nm, RL3 and RL4 are distant from 0.5 to 0.8 nm, L3 and L4 are distant from 0.35 to 0.5 nm, RL4 and RL5 are distant from 0.45 to 0.75 nm, L4 and L5 are distant from 0.4 to 0.55 nm, RL5 and RL6 are distant from 0.4 to 1.2 nm, and L5 and L6 are distant from 0.4 to 0.6 nm, a' in the structure of construction (VI) is such that RL5 and RL6 are distant from 0.4 to 1.2 nm and such that L5 and L6 are distant from 0.4 to 0.6 nm, and b' in the structure of construction (VI) is such that RL6 and RCa4 are distant from 0 to 0.35 nm and such that L6 and LCa4 are distant from 0 to 0.35 nm, wherein the structure may either be closed or open at a and/or at h, where C is a chemical structure with an affinity for a phospholipid, characterized in that it comprises a molecule with the following formula (VII):

$$RL1\text{-}N^1\text{-}RL2\text{-}M\text{-}RL3\text{-}N^2\text{-}RL4\text{-}N^3\text{-}RL5\text{-}RL6 \qquad (VII)$$

wherein N^1 to N^3 each independently represent 1 to 4, independently selected, natural or non-natural, amino acids and wherein M is a peptide consisting of 1 to 100 natural or non-natural amino acids

wherein RL1, RL2, RL3 and RL6 are independently selected from Lys, Arg or Orn; RL4 is independently selected from Asp or Glu; and RL5 is independently selected from Ser, Thr, Asp, or Glu, wherein said structure is linear or cyclic, where D is a chemical structure with an affinity for a phospholipid, characterized in that it comprises at least a portion of a peptide sequence selected from ID No.1 sequence shown in Fig. 6a, ID No.2 sequence

shown in Fig. 6b, ID No.3 sequence shown in Fig. 6c, and ID No.4 and No.5 sequences shown in Fig. 6d or a modified sequence of the latter, where E is a chemical structure with an affinity for a negatively charged phospholipid, characterized in that it comprises a cyclic peptide sequence of the following formula (VIII):

Gly-RL1-Phe-RL2-P^1-Gly-Tyr-P^2-RL3-P^3-RL4-Q^1-RL5-Trp-RL6

(VIII)

wherein RL1 and RL6 are independently selected from Lys, Orn and Arg; RL2 and RL3 are Arg; RL4 and RL5 are independently selected from Asp and Glu;

wherein P^1, P^2 and P^3 are independently selected from Ser and Thr; wherein Q^1 is selected from Gly and Met, said structures being bound.

22. (Amended) A chemical assembly according to claim 21, wherein at least selected from the group consisting of A, B, C, D and E.

23. (Amended) A method for producing a chemical structure as defined in Claim 10, preparing a cDNA comprising a coding sequence of bases for said chemical structure, inserting the cDNA in an appropriate expression vector, transforming an appropriate host cell for replicating the plasmid and producing said structure by translation of said cDNA.

26. (Amended) The method according to claim 23, wherein the appropriate host cell is *E. Coli*.

27. (Amended) A pharmaceutical composition comprising a chemical structure as defined in Claim 1.

28. (Amended) A pharmaceutical composition comprising a chemical assembly as defined in Claim 21.

29. (Amended) A method of treating a thrombosis, tumor or inflammation with the pharmaceutical composition claimed in Claim 27.

30. (Amended) A method for producing a material for covering thrombogenic biomaterial comprising incorporating a structure as claimed in Claim 1.

31. (Amended) A labelling compound comprising a structure as defined in Claim 1 coupled with a labelling molecule.

32. (Amended) A labelling compound comprising an assembly as defined in claim 21 coupled with a labelling molecule.

33. (Amended) The compound according to claim 31, wherein the labelling molecule is selected from a fluorescent molecule, an avidin-biotin complex, a radioelement, and a paramagnetic compound.

34. (Amended) A diagnostic kit comprising a compound according to Claim 31.

36. (Amended) A kit for analyzing and detecting negative charges at the surface of cells, comprising a structure according to Claim 1, coupled with a tracer.

37. (Amended) A kit for analyzing and detecting negative charges at the surface of cells, comprising an assembly according to Claim 21, coupled with a tracer.

38. (Amended) A kit for analyzing and detecting microvesicles in blood at the surface of cells, comprising a structure according to Claim 1, coupled with a tracer.

39. (Amended) A kit for analyzing and detecting microvesicles in blood at the surface of cells, comprising an assembly according to Claim 21, coupled with a tracer.

Please add new Claims 40-115 as follows:

40. (New) The chemical structure according to any of claim 2, wherein the platform is a portion of a domain of the annexin or of a modified domain of the annexin, comprising at least said residual ligands, RL1 to RL6, having said functions L1 to L6 for binding to the phospholipid respectively.

41. (New) The chemical structure according to claim 40, wherein the annexin domain is selected from the domain 1 of annexin V shown in Fig. 6b, domain 2 of annexin I shown in Fig. 6a, domain 2 of annexin III shown in Fig. 6c and domain 1 and 2 of annexin IV shown in Fig. 6d.

42. (New) The chemical structure according to claim 41, wherein the residual ligands RL1 to RL6 respectively are either the residues Arg25, Lys29, Arg63, Asp68, Ser71 and Glu72 of domain 1 of annexin V shown in Fig. 6b or residues Arg124, Lys128, Arg162, Asp167, Ser170 and Asp171 of domain 2 of annexin I shown in Fig. 6a, or residues Lys100, Lys104, Lys138, Asp143, Ser146 and Glu147 of domain 2 of annexin III shown in Fig. 6c, or residues Arg96, Lys101, Arg135, Asp140, Ser143 and Asp144 of domain 2 of annexin IV shown in Fig. 6d, or residues Arg24, Lys28, Arg62, Asp67, Ser70 and Glu71 of domain 1 of annexin IV shown in Fig. 6d.

43. (New) The chemical structure according to claim 17, further comprising a calcium site where the calcium ion is complexed by this site forms one of the ligands of the negatively charged phospholipid.

44. (New) The chemical structure according to Claim 2, said structures having an affinity for a phospholipid selected from a phosphatidylserine, a phosphatidylethanolamine, a phosphatidylinositol, a phosphatidic acid, and a cardiolipid.

45. (New) The chemical structure according to Claim 13, said structures having an affinity for a phospholipid selected from a phosphatidylserine, a phosphatidylethanolamine, a phosphatidylinositol, a phosphatidic acid, and a cardiolipid.

46. (New) The chemical structure according to Claim 17, said structures having an affinity for a phospholipid selected from a phosphatidylserine, a phosphatidylethanolamine, a phosphatidylinositol, a phosphatidic acid, and a cardiolipid.

47. (New) The chemical structure according to Claim 18, said structures having an affinity for a phospholipid selected from a phosphatidylserine, a phosphatidylethanolamine, a phosphatidylinositol, a phosphatidic acid, and a cardiolipid.

48. (New) A method for producing a chemical structure as defined in Claim 11, comprising preparing a cDNA comprising a coding sequence of bases for said chemical structure, inserting the cDNA in an appropriate expression vector, transforming an appropriate host cell for replicating the plasmid and producing said structure by translation of said cDNA.

49. (New) A method for producing a chemical structure as defined in Claim 12, comprising preparing a cDNA comprising a coding sequence of bases for said chemical structure, inserting the cDNA in an appropriate expression vector, transforming an appropriate host cell for replicating the plasmid and producing said structure by translation of said cDNA.

50. (New) A method for producing a chemical structure as defined in Claim 13, comprising preparing a cDNA comprising a coding sequence of bases for said chemical structure, inserting the cDNA in an appropriate expression vector, transforming an appropriate host cell for replicating the plasmid and producing said structure by translation of said cDNA.

51. (New) A method for producing a chemical structure as defined in Claim 17, comprising preparing a cDNA comprising a coding sequence of bases for said chemical structure, inserting the cDNA in an appropriate expression vector, transforming an appropriate host cell for replicating the plasmid and producing said structure by translation of said cDNA.

52. (New) A method for producing a chemical structure as defined in Claim 18, comprising preparing a cDNA comprising a coding sequence of bases for said chemical structure, inserting the cDNA in an appropriate expression vector, transforming an appropriate host cell for replicating the plasmid and producing said structure by translation of said cDNA.

53. (New) A method for producing a chemical structure as defined in Claim 48, characterized in that it comprises steps consisting of preparing a cDNA comprising a coding sequence of bases for said chemical structure, inserting the cDNA in an appropriate expression vector, transforming an appropriate host cell for replicating the plasmid and producing said structure by translation of said cDNA.

54. (New) A method for producing a chemical structure as defined in Claim 49, comprising preparing a cDNA comprising a coding sequence of bases for said chemical structure, inserting the cDNA in an appropriate expression vector, transforming an appropriate host cell for replicating the plasmid and producing said structure by translation of said cDNA.

55. (New) A method for producing a chemical structure as defined in Claim 50, comprising preparing a cDNA comprising a coding sequence of bases for said chemical structure, inserting the cDNA in an appropriate expression vector, transforming an

appropriate host cell for replicating the plasmid and producing said structure by translation of said cDNA.

56. (New) A method for producing a chemical structure as defined in Claim 51, comprising preparing a cDNA comprising a coding sequence of bases for said chemical structure, inserting the cDNA in an appropriate expression vector, transforming an appropriate host cell for replicating the plasmid and producing said structure by translation of said cDNA.

57. (New) A method for producing a chemical structure as defined in Claim 52, comprising preparing a cDNA comprising a coding sequence of bases for said chemical structure, inserting the cDNA in an appropriate expression vector, transforming an appropriate host cell for replicating the plasmid and producing said structure by translation of said cDNA.

58. (New) The method according to Claim 48, wherein the vector is a pGEX-2T vector.

59. (New) The method according to Claim 49, wherein the vector is a pGEX-2T vector.

60. (New) The method according to Claim 50, wherein the vector is a pGEX-2T vector.

61. (New) The method according to Claim 51, wherein the vector is a pGEX-2T vector.

62. (New) The method according to Claim 52, wherein the vector is a pGEX-2T vector.

63. (New) The method according to claim 48, wherein the appropriate host cell is *E. Coli*.

64. (New) The method according to claim 49, wherein the appropriate host cell is *E. Coli.*

65. (New) The method according to claim 50, wherein the appropriate host cell is *E. Coli.*

66. (New) The method according to claim 51, wherein the appropriate host cell is *E. Coli.*

67. (New) The method according to claim 52, wherein the appropriate host cell is *E. Coli.*

68. (New) A pharmaceutical composition comprising a chemical structure as defined in Claim 2 and an inert material.

69. (New) A pharmaceutical composition comprising a chemical structure as defined in Claim 13 and an inert material.

70. (New) A pharmaceutical composition comprising a chemical structure as defined in Claim 17 and an inert material.

71. (New) A pharmaceutical composition comprising a chemical structure as defined in Claim 18 and an inert material.

72. (New) A pharmaceutical composition comprising a chemical structure as defined in Claim 22 and an inert material.

73. (New) A method of treating a thrombosis, tumor or inflammation with the pharmaceutical composition claimed in Claim 68.

74. (New) A method of treating a thrombosis, tumor or inflammation with the pharmaceutical composition claimed in Claim 69.

75. (New) A method of treating a thrombosis, tumor or inflammation with the pharmaceutical composition claimed in Claim 70.

76. (New) A method of treating a thrombosis, tumor or inflammation with the pharmaceutical composition claimed in Claim 71.

77. (New) A method of treating a thrombosis, tumor or inflammation with the pharmaceutical composition claimed in Claim 28.

78. (New) A method of treating a thrombosis, tumor or inflammation with the pharmaceutical composition claimed in Claim 72.

79. (New) A method for producing a material for covering thrombogenic biomaterial comprising incorporating a structure as claimed in Claim 2.

80. (New) A method for producing a material for covering thrombogenic biomaterial comprising incorporating a structure as claimed in Claim 13.

81. (New) A method for producing a material for covering thrombogenic biomaterial comprising incorporating a structure as claimed in Claim 17.

82. (New) A method for producing a material for covering thrombogenic biomaterial comprising incorporating a structure as claimed in Claim 18.

83. (New) A labelling compound comprising a structure as defined in Claim 2 coupled with a labelling molecule.

84. (New) A labelling compound comprising a structure as defined in Claim 13 coupled with a labelling molecule.

85. (New) A labelling compound comprising a structure as defined in Claim 17 coupled with a labelling molecule.

86. (New) A labelling compound comprising a structure as defined in Claim 18 coupled with a labelling molecule.

87. (New) A labelling compound comprising an assembly as defined in claim 22 coupled with a labelling molecule.

88. (New) The compound according to Claim 83, wherein the labelling molecule is selected from a fluorescent molecule, the avidin-biotin complex, a radioelement, and a paramagnetic compound.

89. (New) The compound according to Claim 84, wherein the labelling molecule is selected from a fluorescent molecule, the avidin-biotin complex, a radioelement, and a paramagnetic compound.

90. (New) The compound according to Claim 85, wherein the labelling molecule is selected from a fluorescent molecule, the avidin-biotin complex, a radioelement, and a paramagnetic compound.

91. (New) The compound according to Claim 86, wherein the labelling molecule is selected from a fluorescent molecule, the avidin-biotin complex, a radioelement, and a paramagnetic compound.

92. (New) The compound according to Claim 32, wherein the labelling molecule is selected from a fluorescent molecule, the avidin-biotin complex, a radioelement, and a paramagnetic compound.

93. (New) The compound according to Claim 87, wherein the labelling molecule is selected from a fluorescent molecule, the avidin-biotin complex, a radioelement, and a paramagnetic compound.

94. (New) A diagnostic kit comprising a compound according to Claim 83.

95. (New) A diagnostic kit comprising a compound according to Claim 84.

96. (New) A diagnostic kit comprising a compound according to Claim 85.

97. (New) A diagnostic kit comprising a compound according to Claim 86.

98. (New) A diagnostic kit comprising a compound according to Claim 32.

99. (New) A diagnostic kit comprising a compound according to Claim 87.

100. (New) The diagnostic kit according to Claim 94, further comprising an adequate reagent enabling said labelling molecule to be detected.

101. (New) The diagnostic kit according to Claim 95, further comprising an adequate reagent enabling said labelling molecule to be detected.

102. (New) The diagnostic kit according to Claim 96, further comprising an adequate reagent enabling said labelling molecule to be detected.

103. (New) The diagnostic kit according to Claim 97, further comprising an adequate reagent enabling said labelling molecule to be detected.

104. (New) The diagnostic kit according to Claim 99, further comprising an adequate reagent enabling said labelling molecule to be detected.

105. (New) The diagnostic kit according to Claim 99, further comprising an adequate reagent enabling said labelling molecule to be detected.

106. (New) A kit for analyzing and detecting negative charges at the surface of cells, comprising a structure according to Claim 2, coupled with a tracer.

107. (New) A kit for analyzing and detecting negative charges at the surface of cells, comprising a structure according to Claim 13, coupled with a tracer.

108. (New) A kit for analyzing and detecting negative charges at the surface of cells, comprising a structure according to Claim 17, coupled with a tracer.

109. (New) A kit for analyzing and detecting negative charges at the surface of cells, comprising a structure according to Claim 18, coupled with a tracer.

110. (New) A kit for analyzing and detecting negative charges at the surface of cells, comprising an assembly according to Claim 22, coupled with a tracer.

111. (New) A kit for analyzing and detecting microvesicles in blood at the surface of cells, comprising a structure according to Claim 2, coupled with a tracer.

112. (New) A kit for analyzing and detecting microvesicles in blood at the surface of cells, characterized in that it comprises a structure according to Claim 13, coupled with a tracer.

113. (New) A kit for analyzing and detecting microvesicles in blood at the surface of cells, characterized in that it comprises a structure according to Claim 17, coupled with a tracer.

114. (New) A kit for analyzing and detecting microvesicles in blood at the surface of cells, characterized in that it comprises a structure according to Claim 18, coupled with a tracer.

115. (New) A kit for analyzing and detecting microvesicles in blood at the surface of cells, characterized in that it comprises an assembly according to Claim 22, coupled with a tracer.

REMARKS

Claims 1-115 are active in the present application. Claims 10, 15, 19-23, 26-34 and 36-39 have been amended to remove multiple dependencies and for clarity. Claims 40-114 are new claims. Support for new Claims 40-115 are found in Claims 1-39. No new matter is added. An action on the merits and allowance of claims is solicited.

Respectfully submitted,

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Serial No: 09/787,923
Amendment Filed on:
6-25-01

10. (Amended) The chemical structure according to [any of claims 1 to 9] Claim 1, wherein the platform is a portion of a domain of the annexin or of a modified domain of the annexin, comprising at least said residual ligands, RL1 to RL6, having said functions L1 to L6 for binding to the phospholipid respectively.

15. (Amended) The chemical structure according to Claim 13 [or 14], wherein M is a peptide consisting of 33 natural or non-natural amino acids.

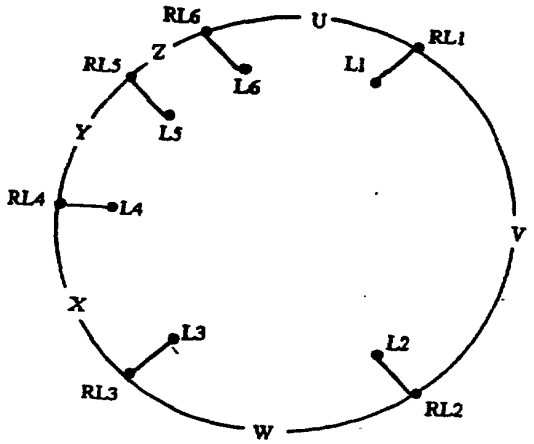
19. (Amended) The chemical structure according to [any of claims 13 to 17] Claim 13, further comprising a calcium site where the calcium ion is complexed by this site forms one of the ligands of the negatively charged phospholipid.

20. (Amended) The chemical structure according to [any of the preceding claims] Claim 1, said structures having an affinity for a phospholipid selected from a phosphatidylserine, a phosphatidylethanolamine, a phosphatidylinositol, a phosphatidic acid, and a cardiolipid.

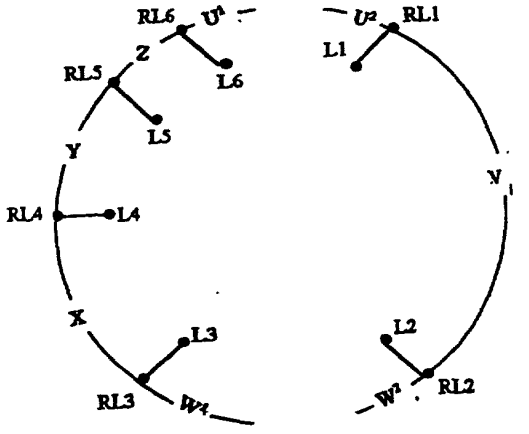
21. (Amended) A chemical assembly having an affinity for a phospholipid, [characterized in that it comprises] comprising at least two identical or different chemical structures [defined in claims 1 to 20] selected from the group consisting of A, B, C, D and E,

where A is a chemical structure with an affinity for a phospholipid, consisting of at least a chemical platform U, V, W, X, Y, Z including six residues RL1, RL2, RL3, RL4, RL5, RL6 supporting a set of chemical functions which may bind to said phospholipid,

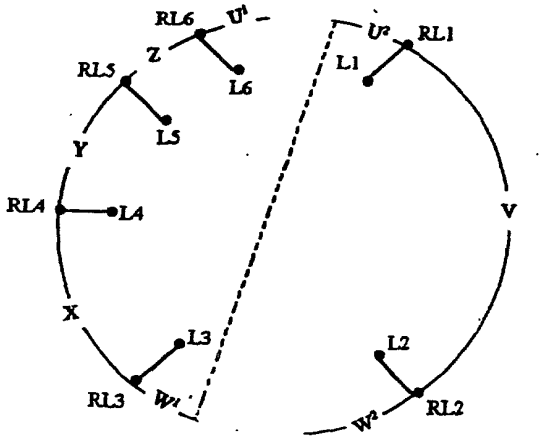
called, L1, L2, L3, L4, L5, L6 respectively, wherein these chemical functions L define the affinity of said structure for said phospholipid, said structure having one of the following constructions (I), (II) and (III):



(I)



(II)



(III)

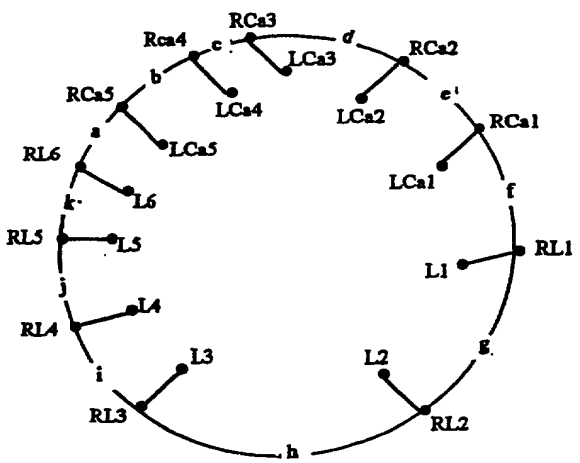
wherein U, U^1, U^2, V, W, W^1, W^2, X, Y, Z are independently a natural or non-natural amino-acid, a peptide consisting of natural or non-natural amino-acids, a carbon chain, or carbon cyclic group(s),

wherein RL1 to RL6 are selected from molecules having the binding chemical functions L1 to L6, respectively, wherein said chemical functions comprise either at least a positive charge, donor of a hydrogen bond, or at least a negative charge, acceptor of a hydrogen bond, and

wherein U, U^1, U^2, V, W, W^1, W^2, X, Y, Z are such that RL6 and RL1 are distant from 0.65 to 0.95 nm, L6 and L1 are distant from 0.65 to 0.9 nm, RL1 and RL2 are distant from 0.45 to 0.65 nm, L1 and L2 are distant from 0.4 to 0.55 nm, RL2 and RL3 are distant from 0.5 to 1.05 nm, L2 and L3 are distant from 0.4 to 0.6 nm, RL3 and RL4 are distant from 0.5 to 0.8 nm, L3 and L4 are distant from 0.35 to 0.5 nm, RL4 and RL5 are distant from 0.45 to

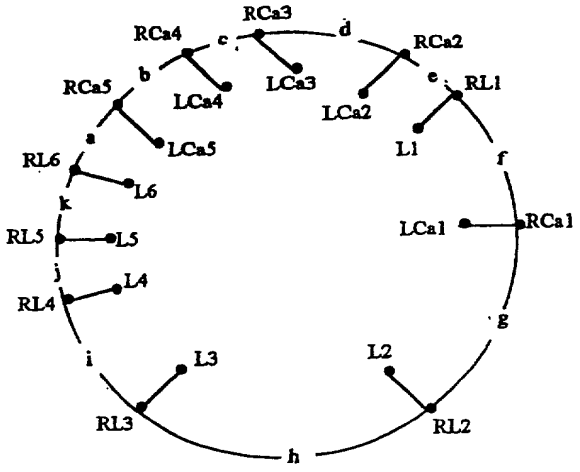
0.75 nm, and L4 and L5 are distant from 0.4 to 0.55 nm, RL5 and RL6 are distant from 0.4 to 1.2 nm, L5 and L6 are distant from 0.4 to 0.6 nm,

where B is a chemical structure with an affinity for a phospholipid, consisting of at least a chemical platform a, a', b, b', c, d, e, f, g, h, i, j, k, l including 11 residues, LR1, LR2, LR3, LR4, LR5, RL6, RCa1, RCa2, RCa3, RCa4 and RCa5 supporting a set of chemical functions which may bind to said phospholipid called L1, L2, L3, L4, L5, L6, respectively, and a set of chemical functions binding to a calcium atom called LCa1, LCa2, LCa3, LCa4, LCa5, respectively, wherein these chemical functions RL1 to RCa5 define the affinity of said structure for said phospholipid, said structure having one of the following constructions (IV), (V) and (VI):

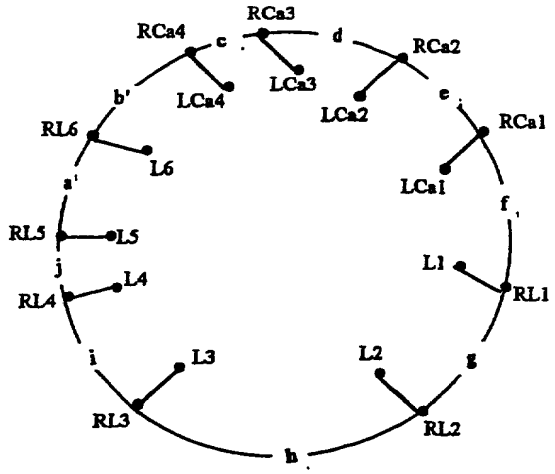


(IV)

wherein a, a', b, b', c, d, e, f, g, h, i, j, k, l, are independently a natural or non-natural amino acid, a peptide consisting of natural or non-natural amino acids, a carbon chain, or carbon cyclic group(s),


RCa3
RCa4
c
d
RCa2
b
e
RCa5
LCa3
RL1
LCa4
LCa2
a
L1
LCa5
f
RL6
L6
k
LCa1
RCa1
RL5
L5
j
L4
RL4
g
i
L3
L2
RL3
RL2
h

(V)


RCa3
RCa4
c
d
RCa2
LCa3
b'
LCa4
LCa2
e
RL6
RCa1
L6
LCa1
a'
f
RL5
L5
j
L1
L4
RL1
RL4
i
L3
L2
g
RL3
RL2
h

(VI)

wherein RL1 to RL6 and RCa1 to RCa5 are selected from molecules having chemical binding functions L1 to L6 and LCa1 to LCa5, respectively, wherein said chemical functions L1 to L6 comprise either at least a positively charged donor of a hydrogen bond, or at least a negatively charged acceptor of a hydrogen bond, said chemical functions LCa1 to LCa5 comprising an oxygen atom, and

wherein a in the structures of construction (IV) and (V) is such that RL6 and RCa5 are distant from 0 to 0.35 nm and such that L6 and LCa5 are distant from 0 to 0.3 nm, b in the structures of construction (IV) and (V) is such that RCa5 and RCa4 are distant from 0 to 0.35 nm and such that LCa5 and LCa4 are distant from 0.2 to 0.3 nm, b' in the structure of construction (VI) is such that RL6 and RCa4 are distant from 0 to 0.35 nm and such that L6 and LCa4 are distant from 0 to 0.35 nm, c and d are such that RCa4 and RCa3 are distant from 0.5 to 0.9 nm, LCa4 and LCa3 are distant from 0.2 to 0.4 nm, RCa3 and RCa2 are distant from 0.35 to 0.6 nm, and LCa3 and LCa2 are distant from 0.22 to 0.3 nm, e, f, g, in the structures of construction (IV), (V), (VI) are such that RL1 and RL2 are distant from 0.45 to 0.65 nm, RCa1 to RCa2 are distant from 0.4 to 0.55 nm, L1 and L2 are distant from 0.4 to 0.55 nm and LCa1 and LCa2 are distant from 0.3 to 0.4 nm, h, i, j and k are such that RL2 and RL3 are distant from 0.5 to 1.05 nm, L2 and L3 are distant from 0.4 to 0.6 nm, RL3 and RL4 are distant from 0.5 to 0.8 nm, L3 and L4 are distant from 0.35 to 0.5 nm, RL4 and RL5 are distant from 0.45 to 0.75 nm, L4 and L5 are distant from 0.4 to 0.55 nm, RL5 and RL6 are distant from 0.4 to 1.2 nm, and L5 and L6 are distant from 0.4 to 0.6 nm, a' in the structure of construction (VI) is such that RL5 and RL6 are distant from 0.4 to 1.2 nm and such that L5 and L6 are distant from 0.4 to 0.6 nm, and b' in the structure of construction (VI)

is such that RL6 and RCa4 are distant from 0 to 0.35 nm and such that L6 and LCa4 are distant from 0 to 0.35 nm, wherein the structure may either be closed or open at a and/or at h,

where C is a chemical structure with an affinity for a phospholipid comprising a molecule with the following formula (VII):

$$RL1\text{-}N^1\text{-}RL2\text{-}M\text{-}RL3\text{-}N^2\text{-}RL4\text{-}N^3\text{-}RL5\text{-}RL6 \quad (VII)$$

wherein N^1 to N^3 each independently represent 1 to 4, independently selected, natural or non-natural, amino acids and wherein M is a peptide consisting of 1 to 100 natural or non-natural amino acids

wherein RL1, RL2, RL3 and RL6 are independently selected from Lys, Arg or Orn; RL4 is independently selected from Asp or Glu; and RL5 is independently selected from Ser, Thr, Asp, or Glu, wherein said structure is linear or cyclic,

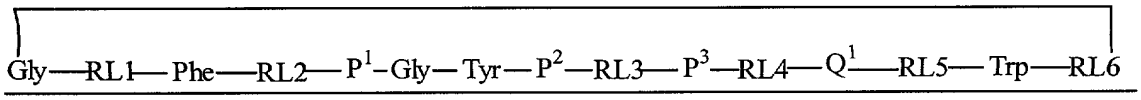
where D is a chemical structure with an affinity for a phospholipid, comprising at least a portion of a peptide sequence selected from ID No.1 sequence shown in Fig. 6a, ID No.2 sequence shown in Fig. 6b, ID No.3 sequence shown in Fig. 6c, and ID No.4 and No.5 sequences shown in Fig. 6d or a modified sequence of the latter,

where E is a chemical structure with an affinity for a negatively charged phospholipid, comprising a cyclic peptide sequence of the following formula (VIII):

wherein RL1 and RL6 are independently selected from Lys, Orn and Arg; RL2 and RL3 are Arg; RL4 and RL5 are independently selected from Asp and Glu;

wherein P^1, P^2 and P^3 are independently selected from Ser and Thr; wherein Q^1 is selected from Gly and Met,

said structures being bound.



(VIII)

22. (Amended) A chemical assembly according to claim [23] 21, wherein at least one of the chemical structures is [one of the chemical structures defined in claims 13 to 20] selected from the group consisting of A, B, C, D and E.

23. (Amended) A method for producing a chemical structure as defined in [any of the preceding claims 10 to 20, characterized in that it comprises steps consisting of] Claim 10, comprising preparing a cDNA comprising a coding sequence of bases for said chemical structure, inserting the cDNA in an appropriate expression vector, transforming an appropriate host cell for replicating the plasmid and producing said structure by translation of said cDNA.

26. (Amended) The method according to claim 23, [24 or 25] wherein the appropriate host cell is *E. Coli*.

27. (Amended) [A use of] A pharmaceutical composition comprising a chemical structure as defined in [claims 1 to 20 for preparing a drug] Claim 1 and an inert material.

28. (Amended) [A use of] A pharmaceutical composition comprising a chemical assembly as defined in [claims 21 or 22 for preparing a drug] Claim 21 and an inert material.

29. (Amended) [The use according to claim 27 or 28, wherein the drug is selected from a drug for treating a thrombosis, a drug for treating a tumor, a drug with an anti-inflammatory action] A method of treating a thrombosis, tumor or inflammation with the pharmaceutical composition claimed in Claim 27.

30. (Amended) [A use of a structure as defined in claims 1 to 19] A method for producing a material for covering thrombogenic biomaterial comprising incorporating a structure as claimed in Claim 1.

31. (Amended) A labelling compound [characterized in that it comprises] comprising a structure as defined in [claims 1 to 20] Claim 1 coupled with a labelling molecule.

32. (Amended) A labelling compound [characterized in that it comprises] comprising an assembly as defined in claim 21 [or 22] coupled with a labelling molecule.

33. (Amended) The compound according to claim 31 [or 32], wherein the labelling molecule is selected from a fluorescent molecule, [the] an avidin-biotin complex, a radioelement, and a paramagnetic compound.

34. (Amended) A [diagnose] diagnostic kit comprising a compound according to [any of claims 31 to 32] Claim 31.

36. (Amended) A kit for analyzing and detecting negative charges at the surface of cells, [characterized in that it comprises] comprising a structure according to [any of claims 1 to 20] Claim 1, coupled with a tracer.

37. (Amended) A kit for analyzing and detecting negative charges at the surface of cells, [characterized in that it comprises] comprising an assembly according to [any of claims 21 or 22] Claim 21, coupled with a tracer.

38. (Amended) A kit for analyzing and detecting microvesicles in blood at the surface of cells, [characterized in that it comprises] comprising a structure according to [any of claims 1 to 20] Claim 1, coupled with a tracer.

39. (Amended) A kit for analyzing and detecting microvesicles in blood at the surface of cells, [characterized in that it comprises] comprising an assembly according to [any of claims 21 to 22] Claim 21, coupled with a tracer.

Claims 48-115 (New).

CHEMICAL STRUCTURE HAVING AN AFFINITY FOR A PHOSPHOLIPID AND LABELLING COMPOUND, DIAGNOSE KIT, AND DRUG COMPRISING THIS STRUCTURE

Technical field

The present invention relates to a chemical structure having an affinity for a phospholipid as well as to a detection molecule, to a conjugate and to a pharmaceutical composition comprising said structure.

Generally speaking, the chemical structure of the present invention is useful for specific recognition of lipid vectors. It may be used for engineering and generation of compounds for recognizing and sequestrating lipids, notably negatively charged lipids, such as phosphatidylserine and/or phosphatidic acid.

These lipids play an important role notably in cellular signaling and they may be present at the external surface of cell membranes and/or circulate in the blood medium following very diverse pathological events.

Diverse cellular events result in the occurrence of phosphatidylserine (PS) at the external cell surface, whereby these events may result either from an accidental or pathological alteration of the cell, or from a programmed cellular event such as cell death or apoptosis. Occurrence of PS at the external surface of cells therefore forms an important "primary message" revealing the existence of a malfunction. In the case of a blood coagulation process, the mechanism is well

described: the alteration of the endothelial cells of the blood vessels, either for accidental reasons or for more complex pathological reasons, causes the occurrence of this PS message at the external surface of cells in contact with the blood medium. This message is immediately recognized by certain circulating proteins which then trigger a cascade of events resulting in the well-known blood coagulation phenomenon.

The invention makes the most of the property of the structure which it provides of binding itself, whether in presence of calcium or not, to lipids and notably to negatively charged ones, for developing useable compounds as research, diagnose and therapeutic tools in the field of recognition of lipid effectors as a rule and of detection of apoptosis, blood coagulation disorders, of septic chock and in particular acute inflammatory pathologies.

As regards research and diagnose, the structure of the invention may for example be coupled with detection molecules, for example a fluorescent molecule, the avidin-biotin complex, a short-lived radioelement or a paramagnetic compound. For instance, with these detection molecules, it is possible to detect apoptotic cells or to recognize negatively charged membrane microdomains.

The structure of the present invention may therefore be used for "*in vitro*" detection of pathologies involving occurrence of negative charges at the surface of the cells and release of microvesicles

into the blood.

The structure of the present invention may also be used when it is coupled for example with a short lived radioelement, for "*in vitro*" detection of thrombotic areas upon vascular, in particular cerebro-vascular accidents of any kind, by using imaging systems. This structure may further be used when it is coupled with a paramagnetic compound such as a gadolinium complex for "*in vivo*" detection of thrombotic, in particular brain areas, by using magnetic resonance imaging (MRI).

As for therapeutics, generally speaking, the structure of the present invention may be used by itself or coupled with a therapeutic molecule in order to prepare a drug which may for example be used orally. For example, such a drug may be used for targeting this molecule towards areas having negative charges such as tumors having centers of apoptotic cells or inflammatory tumors.

The structure of the present invention may for example be coupled with thrombolytic action molecules in order to prepare a drug which may be for example used orally as an anticoagulant in the treatment and prophylaxis of thrombosis or to prepare a molecule covering all thrombogenic biomaterials. The structure of the present invention may therefore be used for targeting thrombolytic molecules at the site of the thrombus or towards the thrombogenic areas.

In another exemplary application of the present invention, the structure of the invention may be used by itself or coupled with an anti-inflammatory molecule

in order to prepare a drug which may be used orally, for example in acute pathologies like asthma, haemorrhagic rectocolitis (HRC), Crohn's disease, septic shock, collagenosis and arthritis.

State of the art

A family of proteins, called annexins, have been described in the prior art as having reversible functional anchoring to the cell membrane, controlled by the calcium concentration and the presence of anionic phospholipids. Annexins form a protein family expressed in very diverse tissues, both in animals and in plants. It seems that they are neither expressed in bacteria nor in yeasts.

The structure of annexins includes four domains of about 70 amino acids or residues, very fairly homologous in sequence but of a nearly identical topology.

Appended Fig. 1A is a diagram of the general topology of an annexin and appended Fig. 1B is a diagram of the topology of a domain of annexin bearing a calcium site. In Fig. 1A, C represents the C-terminal end of this protein, N represents the N-terminal end of this protein. Domains, noted as D1-D4, are associated in two modules, a covalent one D2D3, and the other, a non-covalent one D1D4. In Fig. 1B, A represents a first α helix, B represents a third α helix, D represents a fourth α helix, E represents a fifth α helix and Ca represents the calcium atom. Association of these

helices forms the consensus structure for an annexin domain.

Presently, their biological roles still remain undefined.

In document WO 92/19279, J. Tait describes conjugates having an affinity for phospholipids. In particular, he describes the use of annexin, in particular annexin V, for producing an active conjugate usable as a thrombolytic agent.

Unfortunately, the described conjugate in this document is prepared from entire annexin by a genetic recombination method. Consequently, a great number of drawbacks occur, notably a low yield, a high production cost and a fragile conjugate is obtained because of its complex protein portion.

Description of the invention

Specifically, the object of the present invention is to provide a chemical structure having a specific affinity with a phospholipid. The chemical structure of the invention notably has the advantage of being chemically stable and able to be produced in a reproducible way, with a high yield and very reduced production costs as compared with prior art compounds.

The structure of the present invention is characterized in that it comprises at least a chemical platform U, V, W, X, Y including six residues RL1, RL2, RL3, RL4, RL5, RL6 supporting a set of chemical functions which may bind to said phospholipid, called, L1, L2, L3, L4, L5, L6 respectively, whereby these

chemical functions define at least partly the affinity of said structure for said phospholipid, said structure having one of the following constructions (I), (II) and (III):

DESSINS X 3

wherein U, U^1, U^2, V, W, W^1, W^2, X, Y, Z are independently a natural or non-natural amino-acid, a peptide consisting of natural or non-natural amino-acids, a carbon chain, or carbon cyclic group(s),

wherein RL1 to RL6 are selected from the molecules having the binding chemical functions L1 to L6, respectively, wherein said chemical functions comprise either at least a positively charged donor of a hydrogen bond, or at least a negatively charged acceptor of a hydrogen bond, and

wherein U, U^1, U^2, V, W, W^1, W^2, X, Y, Z are such that RL6 and RL1 are distant from 0.65 to 0.95 nm, L6 and L1 are distant from 0.65 to 0.9 nm, RL1 and RL2 are distant from 0.45 to 0.65 nm, L1 and L2 are distant from 0.4 to 0.55 nm, RL2 and RL3 are distant from 0.5 to 1.05 nm, L2 and L3 are distant from 0.4 to 0.6 nm, RL3 and RL4 are distant from 0.5 to 0.8 nm, L3 and L4 are distant from 0.35 to 0.5 nm, RL4 and RL5 are distant from 0.45 to 0.75 nm, and L4 and L5 are distant from 0.4 to 0.55 nm, RL5 and RL6 are distant from 0.4 to 1.2 nm, L5 and L6 are distant from 0.4 to 0.6 nm.

According to the invention, in the structure of constructions (I), (II) or (III), L1, L2, L3 and L6 may

each have at least a positively charged donor of a hydrogen bond, and L4 and L5 may each have at least one negatively charged acceptor of a hydrogen bond.

According to the invention, in the structure of construction (I), (II), or (III), U, V, W, X, Y and Z may be peptides consisting of natural or non-natural amino-acids, and RL1 to RL6 are amino acids selected from a set comprising Lys, Arg, Orn, Ser, Thr, Asp and Glu, or analogs of the latter, L1 to L6 are the charge-bearing functions of the side chains of said amino acids.

According to the invention, in the structure of construction (I), (II) or (III), RL1 to RL6 may be positioned in the space formed by U, V, W, X, Y, Z so that the chemical binding functions L1 to L6 are directly accessible to the phospholipid, from their side chains respectively.

According to the invention, the structures of construction (I), (II) or (III) may further comprise a calcium site where the calcium ion complexed by this site forms one of the ligands of the phospholipid.

The present invention also provides a chemical structure which is characterized in that it comprises at least a chemical platform a, a', b, b', c, d, e, f, g, h, i, j, k, l including 11 residues, LR1, LR2, LR3, LR4, LR5, RL6, RCa1, RCa2, RCa3, RCa4 and RCa5 supporting a set of chemical functions which may bind to said phospholipid, called L1, L2, L3, L4, L5, L6 respectively and a set of chemical functions for binding a calcium atom called LCa1, LCa2, LCa3, LCa4,

LCa5 respectively, wherein these chemical functions RL1 to RCa5 define at least partly the affinity of said structure for said phospholipid, said structure having one of the following constructions (IV), (V) and (VI):

DESSINS X 3

wherein a, a', b, b', c, d, e, f, g, h, i, j, k, l, are independently a natural or non-natural amino acid, a peptide consisting of natural or non-natural amino acids, a carbon chain, or carbon cyclic group(s), wherein LR1 to LR6 and RCa1 to RCa5 are selected from molecules having chemical binding functions L1 to L6 and LCa1 to LCa5 respectively, wherein said chemical functions L1 to L6 comprise either at least a positively charged donor of a hydrogen bond, or at least a negatively charged acceptor of a hydrogen bond, said chemical functions LCa1 to LCa5 comprising an oxygen atom, and

wherein a in the structures of construction (IV) and (V) is such that RL6 and RCa5 are distant from 0 to 0.35 nm and such that L6 and LCa5 are distant from 0 to 0.3 nm, b in the structures of construction (IV) and (V) is such that RCa5 and RCa4 are distant from 0 to 0.35 nm and such that LCa5 and LCa4 are distant from 0.2 to 0.3 nm, b' in the structure of construction (VI) is such that RL6 and RCa4 are distant from 0 to 0.35 nm and such that L6 and LCa4 are distant from 0 to 0.35 nm, c and d are such that RCa4 and RCa3 are distant from 0.5 to 0.9 nm, LCa4 and LCa3 are distant

from 0.2 to 0.4 nm, RCa3 and RCa2 are distant from 0.35 to 0.6 nm, and LCa3 and LCa2 are distant from 0.22 to 0.3 nm, e, f, g, in the structures of construction (IV), (V), (VI) are such that RL1 and RL2 are distant from 0.45 to 0.65 nm, RCa1 to RCa2 are distant from 0.4 to 0.55 nm, L1 and L2 are distant from 0.4 to 0.55 nm and LCa1 and LCa2 are distant from 0.3 to 0.4 nm, h, i, j and k are such that RL2 and RL3 are distant from 0.5 to 1.05 nm, L2 and L3 are distant from 0.4 to 0.6 nm, RL3 and RL4 are distant from 0.5 to 0.8 nm, L3 and L4 are distant from 0.35 to 0.5 nm, RL4 and RL5 are distant from 0.45 to 0.75 nm, L4 and L5 are distant from 0.4 to 0.55 nm, RL5 and RL6 are distant from 0.4 to 1.2 nm, and L5 and L6 are distant from 0.4 to 0.6 nm, a' in the structure of construction (VI) is such that RL5 and RL6 are distant from 0.4 to 1.2 nm and such that L5 and L6 are distant from 0.4 to 0.6 nm, and b' in the structure of construction (VI) is such that RL6 and RCa4 are distant from 0 to 0.35 nm and such that L6 and LCa4 are distant from 0 to 0.35 nm, whereby the structure may be either closed or open at a and/or h.

When the preceding distances a, b, b' are indicated as being possibly zero, it is understood that the two sets (RL6-L6 and RCa5-LCa5) and/or both sets (RCa4-LCa4 and RCa5-LCa5) and or both sets (RL6-L6 and RCa4-LCa4) separately form a single and same set.

The platforms according to the invention consist of a set of structural chemical groups which may comprise a sufficient number of cyclic groups in order

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to provide stiffness compatible with the affinity towards the phospholipid.

The measured distances when RLs and RCas are amino acids, may be measured between the α carbon atoms of these amino acids in the aforementioned structures (I) to (VI).

These structures may be synthesized by conventional synthesis methods of organic chemistry and of protein chemistry, by genetic recombination, by genetic engineering, etc.

Examples of such structures are notably given in " Discovery of Sequence-Selective Peptide Binding by Synthetic Receptors Using Encoded Combinatorial Libraries", W.C. Still, Acc. Chem. Res., 1996, 29, 155-163 and in "Toward Synthetic Adrenaline Receptors: Strong, Selective and Biomimetic Recognition of Biologically Active Amino Alcohols by Bisphosphonate Receptors Molecules", T. Shrader, J. Org. Chem., 1998, 63, 264-272.

According to the invention, in the structure of construction (IV), (V) or (VI), L1, L2, L3 and L6 may each have at least a positively charged donor of a hydrogen bond, and L4, L5, LCa5, LCa4, LCa3, LCa2 and LCa1 may each have at least a negatively charged acceptor of a hydrogen bond.

According to the invention, in the structure of construction (I), (II), (III), RL1, RL2, RL3 and RL6 may be independently selected from Arg, Lys, Orn ; RL4 may be independently selected from Asp or Glu ; and RL5 may be independently selected from

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Ser, Thr, Asp or Glu, whereby the side chains of these amino acids have chemical functions for binding to the phospholipids L1 to L6, respectively.

According to the invention, in the structure of construction (IV), (V) or (VI), a or a', b or b', c, d, e, f, g, h, i, j, k may be peptides consisting of natural or non-natural amino acids, and RL1 to RL5 may be amino acids selected from a set comprising Lys, Arg, Orn, Ser, Thr, Asp and Glu, or analogs thereof, RL6 may be Asp or Glu or analogs of the latter, L1 to L6 and LCa1 to LCa5 may be the charge-bearing functions of the side chains of said amino acids, and RCa1 to RCa5 may be natural or non-natural amino acids.

According to the invention, in the structure of constructions (IV), (V) or (VI), the carbon atoms RL1 to RL6 and RCa1 to RCa2 may be positioned in the space formed by a, b, c, d, e, f g, h, i, j and k so that the chemical binding functions L1 to L6 respectively and the positive charges of the calcium atom when the latter is bound to the bond functions LCa1 to LCa5, are directly accessible to the phospholipid.

According to the invention, in the structure of construction (I), (II), (II), (IV), (V) or (VI), at least a portion of the platform may be a portion of a domain of the annexin or of a modified domain of the annexin, comprising at least one of said residual ligands RL1 to RL6, having said functions L1 to L6 respectively for binding to the phospholipid.

According to the invention, in the structure of construction (I), (II), (III), (IV), (V), or (VI), the platform may be a portion of a domain of the annexin or

a modified annexin domain, wherein said portion of the annexin domain comprises said residual ligands RL1 to RL6 having said functions L1 to L6, respectively.

According to the invention, the annexin domain is selected from the domain 1 of annexin V shown in Fig. 6b, domain 2 of annexin I shown in Fig. 6a, domain 2 of annexin III shown in Fig. 6c and domain 1 and 2 of annexin IV shown in Fig. 6d.

According to the invention, the residual ligands RL1 to RL6 respectively may be either the residues Arg25, Lys29, Arg63, Asp68, Ser71 and Glu72 of domain 1 of annexin V shown in Fig. 6b or residues Arg124, Lys128, Arg162, Asp167, Ser170 and Asp171 of domain 2 of annexin I shown in Fig. 6a, or residues Lys100, Lys104, Lys138, Asp143, Ser146 and Glu147 of domain 2 of annexin III shown in Fig. 6c, or residues Arg97, Lys101, Arg135, Asp140, Ser143 and Asp144 of domain 2 of annexin IV shown in Fig. 6d, or residues Arg24, Lys28, Arg62, Asp67, Ser70 and Glu71 of domain 1 of annexin IV shown in Fig. 6d.

The present invention also provides a chemical structure with an affinity for a phospholipid, characterized in that it comprises a molecule of the following formula (VII):

$$RL1\text{-}N^1\text{-}RL2\text{-}M\text{-}RL3\text{-}N^2\text{-}RL4\text{-}N^3\text{-}RL5\text{-}RL6$$

(VII)

wherein N^1 to N^3 each independently represent 1 to 4 independently selected, natural or non-natural, amino acids and wherein M is a peptide consisting of 1

to 100 natural or non-natural amino acids;

wherein RL1, RL2, RL3 and RL6 are independently selected from Lys, Arg or Orn; RL4 is independently selected from Asp or Glu; and RL5 is independently selected from Ser, Thr, Asp, or Glu, wherein said structure is linear or cyclic.

According to the invention, N^1 may represent three amino acids, N^2 may represent four amino acids, and N^3 may represent two amino acids in the structure of formula VII.

In the structure according to the invention, M may be for example a peptide consisting of 33 natural or non-natural amino acids.

According to the invention, the structure of formula (VII) may be a peptide sequence selected from the peptide sequence from Arg124 to Ser171 in the ID No.1 sequence shown in Fig. 6a, the peptide sequence from Arg25 to Glu72 in the ID No.2 sequence shown in Fig. 6b, the peptide sequence from Lys100 to Glu147 in the ID No.3 sequence shown in Fig. 6c, the sequence from Arg24 to Glu71 in the ID No.4 sequence shown in Fig. 6d, the sequence from Arg97 to Asp144 in ID No.5 sequence shown in Fig. 6d, or a modified sequence of these sequences provided that RL1, RL2, RL3 and RL6 are independently selected from Lys, Arg, or Orn;

RL4 is independently selected from Asp or Glu, and RL5 is independently selected from Ser, Thr, Asp or Glu.

The present invention also provides a chemical structure with an affinity for a phospholipid,

comprising at least a portion of a peptide sequence selected from ID No.1 sequence shown in Fig. 6a, ID No.2 sequence shown in Fig. 6b, ID No.3 sequence shown in Fig. 6c, and ID No.4 and No.5 sequences shown in Fig. 6d or a modified sequence of the latter.

The present invention also provides a chemical structure with an affinity for a negatively charged phospholipid, comprising a cyclic peptide sequence of the following formula (VIII):

Gly-RL1-Phe-RL2-P^1-Gly-Tyr-P^2-RL3-P^3-RL4-Q^1-RL5-Trp-RL6

(VIII)

wherein RL1 and RL6 are independently selected from Lys, Orn and Arg; RL2 and RL3 are Arg; RL4 and RL5 are independently selected from Asp and Glu;

wherein P^1, P^2 and P^3 are independently selected from Ser and Thr; wherein Q^1 is selected from Gly and Met.

The aforementioned chemical structures may further comprise a calcium site where the calcium ion complexed by this site forms one of the ligands of the negatively charged phospholipid. The calcium site may be for example a calcium site analogous to the one of the annexins or phospholipids A2. These calcium sites are known to one skilled in the art.

According to the invention, all the aforementioned chemical structures may have an affinity for a phospholipid selected from a phosphatidylserine, a phosphatidylethanolamine, a phosphatidylinositol, a

phosphatidic acid, and a cardiolipid, the lipid chain(s) of the phospholipids may for example comprise from 4 to 23 carbon atoms. For example, the phospholipid may have a arachidonic acid chain, for example for phosphatidylserine.

The present invention also provides a chemical assembly with an affinity for a phospholipid, comprising at least two of the chemical structures of the present invention, identical or different, wherein said structures are bound.

For example, in a chemical assembly of the present invention, at least one of the chemical structures may be one of the peptide chemical structures described earlier.

The assemblies according to the invention may therefore be composed for example of identical of different structures. For example, the assembly may be an appropriate covalent assembly of two structures according to the invention, for example domains 1 and 4 according to the invention, of a same annexin. This assembly may for example, include a domain 4 according to the invention, modified by genetic engineering for the purpose of introducing a calcium and phospholipid site identical to the one of domain 1 of the invention.

These domains may for example stem from annexins I and V.

These assemblies may notably have the purpose of increasing the affinity of structures of the present invention, for the phospholipid, for example for a negatively charged phospholipid. For example they may

be made by inserting a flexible peptide bond, for example polyglycine, between the chemical structures of the invention.

The structures and assemblies of the present invention exhibit an affinity for phospholipids, and notably for those that are negatively charged, better than 0.1 μM. They may comprise a portion of an annexin or one of its derivatives. This annexin may be a natural annexin or a modified one by conventional chemistry or genetic engineering means.

The present invention also provides a method for producing a chemical structure comprising the steps consisting of preparing a cDNA comprising a coding sequence of bases for said chemical structure, inserting the cDNA in an appropriate expression vector, transforming an appropriate host cell for replicating the plasmid and producing said structure by translation of said cDNA.

According to the invention, in this method, the vector may be a plasmid, for example vector pGEX-2T.

In the method according to the invention, the appropriate host cell may be *E. Coli* for example.

For example, for producing the structure according to the invention, it is possible to start with domain 1 of the annexin I and then modify the sequence in such a way that the RL residues defined earlier and possibly the RCa residues occur in the sequence. Thus, through conventional genetic engineering methods, a coding cDNA for the modified sequence may be produced and the structure of the present invention may be obtained very

easily. The structure according to the invention, when it exhibits at least a peptide portion, may also be produced by a conventional solid phase chemical synthesis method.

An example of the modification of the sequence of domain 1 of the invention of annexin I may consist in replacing His52 with Arg, Met56 with Lys or Arg, Val57 with Gly, Val60 with Thr, possibly Lys90 with Arg, Thr95 with Asp, Lys98 with Ser or Thr, and Ala99 with Asp or Glu. These modifications may also be made on other domains.

These modifications may notably have the role of increasing the general stability of the structure or of the domain as regards temperature, pH, and ionic conditions of the medium used; reducing its possible general toxicity properties towards human organism; increasing its affinity for negatively charged phospholipids; and increasing its general affinity for cell membranes.

According to the invention, the modification of a domain may also have the role of developing the affinity of the structure for a, e.g. negatively charged, phospholipid,; and even of restoring an affinity at least equal to that possessed by so-called wild annexin, in the absence of calcium.

The modification may for example target the residue, the so-called Asp or Glu bidentate residue of calcium (RL6) of the domain(s) bearing a phosphatidylserine site, in order to replace them with one of the Lys or Orn residues.

Another modification, for example of domain 1 of annexin V, may consist in replacing Glu72 with Lys or Orn, and/or Thr33 with Lys or Orn.

According to the invention, the chemical structure or assembly of the present invention may be used for preparing a drug.

For example, the drug may be selected from a drug for treating a thrombosis, a drug for treating a tumor, a drug with an anti-inflammatory action.

According to the invention, the chemical structure or assembly according to the invention may be coupled with a labelling molecule for forming a labelling compound.

According to the invention, the labelling molecule may be selected for example from a fluorescent molecule, the avidin-biotin complex, a radioelement and a paramagnetic compound.

The present invention also provides a diagnose kit comprising an aforementioned structure or assembly.

This diagnose kit may for example further comprise an adequate reagent for detecting said labelling molecule.

The present invention also provides an analysis and detection kit for negative charges at the surface of cells, characterized in that it comprises a chemical structure or assembly of the present invention.

The present invention also provides an analysis and detection kit for microvesicles in blood, characterized in that it comprises a chemical structure or assembly of the present invention coupled with a

tracer.

Other advantages and features of the present invention will further become apparent upon reading the illustrative and non-limiting examples which follow, with reference to the appended figures.

Brief description of the figures

- Fig. 1A is a schematic representation of the general structure of annexins;

- Fig. 1B is a schematic representation of the structure of a domain of an annexin including a calcium site;

- Fig. 2 is diagram illustrating the insertion of coding cDNA for the chemical structure of the present invention into a PGEX-2T vector in order to produce said compound through genetic engineering;

- Fig. 3 is a schematic representation of a ^{1}H NMR spectrum of domain 1 of the present invention of annexin I showing the aliphatic region;

- Fig. 4 is a graphical representation of denaturation of domain 1 of the present invention of annexin I with guanidinium chloride;

- Fig. 5 is a graphical representation of thermal denaturation of domain 1 of the present invention of annexin I;

- Fig. 6a represents the sequence of annexin I, noted as ID No.1 sequence, wherein the sequence of domain 2 of the present invention has been underlined;

- Fig. 6b represents the sequence of annexin V, noted as ID No.2 sequence, wherein the sequence of

domain 1 of the present invention has been underlined;

- Fig. 6c represents the sequence of annexin III, noted as ID No.3 sequence wherein the sequence of domain 2 of the present invention has been underlined;

- Fig. 6d represents the sequence of annexin IV noted as ID No.4 sequence and ID No.5 sequence wherein the sequences of domains 1 and 2 of the present invention have been underlined;

- Fig. 7 is a schematic representation of the structure of construction (I) of the present invention bound to a phosphatidylserine molecule demonstrating the interactions between the binding functions L1 to L6 of the structure of construction (I) of the invention and a phosphatidylserine molecule;

- Fig. 8 is a schematic representation of interactions between the residual ligands of domain 1 of the present invention of human annexin V illustrated in Fig. 6b, and a phosphatidylserine molecule in the presence of a calcium atom;

- Figs. 9A and 9B are photographs of polyacrylamide gels which illustrate the fixing of annexin V and of certain of its mutants on membranes consisting of phosphatidylcholine and phosphatidylserine (supernatant S2).

Examples

Example 1: Expression and purification of peptides with ID No.1 and ID No.2 sequences of the present invention.

ID No.1 and ID No.2 sequences of annexins I and V were prepared by overexpression in *E. Coli* according to

the same protocol as the one described by F. Cordier-Ochsenbein et al. in J. Mol. Biol. 279, 1177-1185.

The cDNA of these annexins sequences was prepared by using PCR from cDNA of the corresponding annexins. The cDNA was inserted into the pGEX-2T vector (Smith & Johnson, 1998). Fig. 2 is a diagram illustrating the insertion of cDNA into the vector. Absence of mutations induced by PCR was controlled by sequencing. Production of the peptide is achieved by using the *E. Coli* BL21 strain containing the expression vector described earlier. After induction by isopropylthiogalacto-pyranoside (IPTG, 100 μm) to an optical density of 1 to 600 nm, growth was continued until a plateau was reached, i.e., for about 3 hours. After centrifugation, bacteria were resuspended in the lysis buffer comprising, 50mM Tris-HCl, pH 8, 10 mM EDTA, 500 mM NaCl, 5% (v/v) glycerol, 1% (n/v) Triton X100, 1mM dithiothreitol (DTT), 1 mM phenylmethylsulfonyl fluoride (PMSF) and 20 μg/ml of aprotinin.

Purification was carried out in the following way: after sonication and centrifugation at 10,000 g, the supernatant containing the soluble proteins is incubated with glutathion/agarose beads providing the bond specific to these beads, of GST domain fusion protein. After washing with a solution containing 1 M NaCl, 50 mM Tris-HCl at pH 8, 70 units of thrombine per liter of culture medium were added and the sequence is eluated.

The sequence is then purified on a proRPC (trade name) column of type 16/10, provided by Pharmacia, by

using a FPLC system and a linear gradient of Millipore (trade name) grade water containing 0.1% (v/v) of trifluoracetic acid TFA, and acetonitrile containing 0.1% of TFA. The flow rate is adjusted to 2.5 ml/mn. The sequence is then freeze-dried. The final yield is about 8 mg of sequence per liter of culture medium.

Example 2: Stability of the ID No.1 sequence of annexin I

Various experiments show that this sequence forms a stable folding protein.

Fig. 3 shows a one-dimensional ^{1}H NMR spectrum of the proton of the ID No.1 sequence isolated from annexin I, in an aqueous solution. Dispersion of resonance frequencies and the presence of resonances at chemical shifts less than 0 ppm clearly show that this sequence is highly structured. Furthermore, the chemical shift data of α protons reveal the presence of 5 helices in agreement with the crystallographic structure.

Fig. 4 shows the cooperative denaturation of domain 1 of annexin I issued from ID No.1 sequence, with guanidinium chloride, which is a standard denaturation agent and Fig. 5 shows the cooperative denaturation of the sequence with temperature.

Analogous data are obtained for the other sequences described earlier and they demonstrate that certain annexin sequences behave like small proteins of normal stability, which may be used directly or as a platform for the engineering of novel functional

compounds.

Example 3-1: The essential role of domain 1 of annexin V issued from ID No.2 sequence in the binding of annexin V to the membranes.

Binding experiments of annexin V to model membrane systems as well as kinase c protein (PKC) *in vitro* and cytoplasmic ($cPLA_2$) phospholipase A_2 (PLA_2) *in vivo* inhibition experiments demonstrate the essential role played by domain 1 in this bonding to membranes.

The case of $cPLA_2$ inhibition is taken here as an example. Inhibition of phospholipasic activity by annexin V results from the depletion of the lipid substrate common to both of these proteins. Various mutants of annexin V were constructed in order to selectively eliminate in one or several domains the calcium bonding capacity, i.e., the phospholipids. The mutation consists of replacing the bidentate ligand of calcium, Glu or Asp, of a sequence of the present invention with a non-binding residue, respectively Gln or Asn. Twelve mutants were thus constructed and purified: M1, M2, M3, M4, M1M2, M1M3, M1M4, M2M3, M1M2M3, M1M2M4, M2M3M4 and M1M2M3M4, the number designating the domain for which the calcium binding capacity is suppressed. All the results show that the phospholipasic activity of cell PLA_2, measured by the desalting rate of arachidonic acid, strongly depends on the presence of the calcium site in domain 1 and to a lesser extent in domain 4. Suppression of calcium sites in domains 2 and 3 has virtually no effect on the

inhibition of phospholipasic activity of $cPLA_2$. (Mira et al. J. Biol. Chem. 1997, 272:10474-10482; Dubois et al. Biochem. J. 1998, 330:1277-1282).

The following Table (I) groups together certain results of this example and shows the percentage of reduction in the binding capacity of mutants from annexin V to phospholipids as compared with wild annexin V.

Wild annexin V	M1	M2	M3	M4	M1M2M3	M1M2M4
0	79±6	38±4	47±9	38±6	98±1	85±7

This table (I) shows the binding of membranes of annexin V and of its mutants M1, M2, M3, M4, M1M2M3 and M1M2M4. Results are expressed as a percentage of the reduction in binding capacity as compared with wild annexin V (mean value ± standard error). For mutants M123 and M124, the residual binding rate is insignificant.

Example 3-2: Preliminary results concerning the binding of annexin V and of various mutants to model membranes consisting of phosphatidylcholine and phosphatidyl-serine

The following mutants of human annexin V were prepared according to the method described in Example 1:

M1M2M3M4: The main calcium site corresponding to

the AB loop, is suppressed in all the domains by a mutation of the bidentate ligand.

M2M3M4: The main calcium site of domains 2, 3 and 4 is suppressed by a mutation of the bidentate ligand, the one of domain 1 subsists.

M2M3M4-Arg22Ala-Arg63Ser: Suppression of ligands L2 and L3 of the PS site of the present invention.

M2M3M4-Arg22Ala-Arg63Ser-Lys29AlaAsp68Ile/Phe/Trp: Suppression of all the ligands of the PS site of the present invention except that those concerning the calcium site are preserved.

The binding capacity of mutants of annexin V to PC/PS membranes is then compared with that of the wild form according to the following protocol:

A homogeneous mixture of PC/PS in a proportion of 80/20 is suspended in solutions containing variable calcium concentrations of 0, 30, 100, 1000 μM. The various proteins are then introduced and incubated for a few minutes. The suspension is then centrifuged by ultra-centrifugation at 90,000 rpm. The membranes settle at the bottom of the tube. The supernatant called S1, is entirely picked up for subsequent analysis of protein content which will provide information on the amount of protein not bound to the membrane. The membrane sediment is then dispersed in a solution containing EDTA in a sufficient amount for desalting the proteins, binding of annexin V being reversible and dependent on calcium. The suspension is again centrifuged and a second supernatant called S2, is recovered. Protein content analysis of S2 provides

information concerning the amount of proteins which are fixed to the membrane.

The analysis of the supernatants is carried out by electrophoresis on polyacrylamide gel in a standard way which does not need to be described herein.

The appended Figs. 9A and 9B show all the results.

In this figure:

Wild: A5 = annexin V

Mutants:

D68F=M2M3M4-Arg22Ala-Arg63Ser-Lys29Ala-Asp68Phe

D68I=M2M3M4-Arg22Ala-Arg63Ser-Lys29Ala-Asp68Ile

D68W=M2M3M4-Arg22Ala-Arg63Ser-Lys29Ala-Asp68Trp

1, 2, 3, 4 = calcium concentration 0, 30, 100, 1,000 μM, respectively

T = molecular mass standards.

Comparison of the behavior of M1M2M3M4 and M2M3M4 mutants with that of wild annexin V clearly shows that virtually the binding to the membranes in presence of calcium is exclusively provided by domain 1, i.e., which contains the claimed PS site. This result confirms those given in Example 3-1 above.

Behavior of mutants M2M3M4-Arg22Ala-Arg63Ser and M2M3M4-Arg22Ala-Arg63Ser-Lys29Ala-Asp68Ile/Phe/Trp shows that the binding to the membranes is considerably attenuated when ligands L2, L3, L4 and L5 are suppressed. However the bond is not totally suppressed to the extent that the LCa5, Ca ligands which are part of the calcium site subsist and still provide a binding of PS but with a very reduced affinity;

Example 4: Use of the chemical structure of the present invention

Three utilization schemes are provided: i) simple engineering of the domains in order to meet various requirements related to their use as research, diagnose and therapeutic tools; ii) redesign of the platform which forms the topology of the domain into a new simpler platform which may be synthetized chemically or through genetic engineering; iii) replacement of the peptide or peptoid platform with a non-peptide organic structure for producing a drug. in the three cases, the purpose is naturally to preserve, or even improve, spatial localization of phospholipid binding functions, as described earlier.

1) Annexin domain engineering

The annexin domains of the present invention form peptide platforms. Modification of the domain's sequence through mutagenesis is understood under the term of engineering, in order to improve the general stability of the molecule and to adapt it to the physico-chemical conditions imposed by its use, to improve its affinity for the phospholipid ligand and to provide it with a specificity, specific to each phospholipid. The aim is also to allow for introduction of various tracers for different applications which are discussed later. Our present knowledge is largely sufficient for carrying out such engineering.

Examples of a change in properties are illustrated in Example 4. They were obtained through a standard

genetic engineering technique with mutation of the involved amino acids.

2) Redesign of peptide platforms

Redesign of the platform consists in redefining a molecular architecture, while maintaining the appropriate topology of the residues involved in the binding to calcium or to phospholipids. The redesign is important for generating a shorter sequence platform which may be produced by chemical synthesis. The synthesis of a peptide of the size of a domain is feasible but remains difficult. However, by reducing the number of residues by half, i.e., about 35 residues, it is currently possible to carry out the synthesis. In this redesign operation, geometry is rather precisely preserved, allowing for interactions with phospholipid and notably for positioning of residues of the annexin sequence. These residues are those shown in bold in Figs. 6a-6d for annexins (I) to (V).

This set comprises two basic residues generally Arg-x-x-x-Lys, at the end of the A helix of the relevant domain and a series of acid, basic and neutral residues, generally Arg-x-x-x-x-Asp-x-x-Ser-Asp, located in the D helix. Study of the molecular structure as in Figs. 7 and 8, shows that these residues are perfectly positioned for binding a phosphatidylserine molecule. The carboxylate group of this lipid is itself bound to the calcium atom in the AB loop and designated in the following as the "AB

calcium site".

The sequence:

Arg-xxx-Lys(helix A)----Arg-x-x-x-x-Asp-x-x-Ser-Asp(helix D)

associated with that of the AB calcium site, is therefore a consensus sequence for the binding of phosphatidylserine in the compounds of the present invention. As a generalization, this sequence will now be designated as:

RL1-x-x-x-RL2----RL3-x-x-x-x-RL4-x-x-RL5-RL6

wherein RL1-RL6 are the essential residual ligands in the phosphatidylserine bond shown in bold in the sequences of Figs. 6a-6d and indicated in the structure compounds (I)-(VI). The consensus sequence of the AB calcium site is the succession:

Met-Lys-*Gly*-x-*Gly*-Thr----*Asp*(or *Glu*)

The calcium ligands are the peptide carboxyl groups of the residues in italics (residues of the AB loop) in the figure and both oxygen atoms of the carboxylate group of the side chain of residue Asp (or Glu) at the end of the D helix, also named as the bidentate ligand. As a generalization, these calcium ligands will now be designated as:

RCa1-RL2-RCa2-x-RCa3-Thr----(RCa4RCa5) or RL6

In the case of annexin, RCa4 and RCa5 form a single and same residue already identified earlier as RL6.

The interatomic distance data between the residual ligands are given in the following Table (II) with reference to appended Fig. 7 and the specific domain-calcium-phosphatidylserine interactions are indicated in the following Table (III) with reference to the appended Fig. 8.

In Fig. 8, Ch1 and Ch2 represent the location of possible carbon chains of the phospholipid. These chains may be the ones described, for example arachidonic acid.

According to the invention, the chemical structure may be formed in the following way:

a) it includes in particular at least 6 residues, so-called residual ligands, named RL1-RL6 and their nature is the following:

RL1 = Arg or Lys or Orn

RL2 = Arg or Lys or Orn

RL3 = Arg or Lys or Orn

RL4 = Asp or Glu

RL5 = Ser or Thr or Asp or Glu

RL6 = Arg or Lys or Orn

b) The α carbon atoms of residual ligands RL1-RL6 are positioned in space so that the side chains are directly accessible to the phospholipids.

c) The α carbon atoms of residual ligands RL1-RL6 are positioned according to the following table of distances (II):

α carbon atom	RL2	RL3	RL4	RL5	RL6
RL1	0.45-0.65	0.7-1.2	0.7-1.0	0.85-1.15	0.65-0.95
RL2		0.5-1.05	0.8-1.2	1.2-1.7	0.9-1.4
RL3			0.5-1.08	1.0-1.3	1.2-1.7
RL4				0.45-0.75	0.7-1.2
RL5					0.4-1.2

d) The side chains of residual ligands RL1-RL6 may establish a network of hydrogen bonds with phosphatidylserine according to the diagram where the arrows → designate at least a hydrogen bond, in Fig. 8, in the direction from donor to acceptor and L1-L6 designate the ligands of phosphatidylserine according to the following list:

L1 = NZLys or CZArg of RL1

L2 = NZLys or CZArg of RL2

L3 = NZLys or CZArg of RL3

L4 = CGAsp or CDGlu of RL4

L5 = CB of Ser or Thr or CG of Asp or CD of Glu of LR5

L6 = NZLys or CZArg of RL6

HN = H

NZ = N zeta

CZ = C zeta

OD = O delta

OG = O gamma

OE = O epsilon

wherein distances between ligands L1-L6 and

phosphatidylserine atoms are given in the following table (III):

Distances nm x 10

	N	Cβ	Cγ	01	02	03	04	C1 Chain Ch1	C1 Chain Ch2
L1	0.35 - 0.65	0.3 - 0.5	0.25 - 0.45	0.2 - 0.35	0.25 - 0.5	0.35 - 0.6	0.2 - 0.35	0.4 - 0.7	0.5 - 0.8
L2	0.55 - 0.85	0.45 - 0.75	0.45 - 0.75	0.4 - 0.6	0.2 - 0.4	0.4 - 0.6	0.25 - 0.45	0.7 - 1.1	0.7 - 1.1
L3	0.4 - 0.6	0.4 - 0.6	0.45 - 0.75	0.4 - 0.6	0.2 - 0.4	0.2 - 0.35	0.25 - 0.5	0.7 - 1.1	0.6 - 1.0
L4	0.25 - 0.45	0.3 - 0.5	0.35 - 0.55	0.55 - 0.85	0.5 - 0.75	0.4 - 0.65	0.4 - 0.6	0.8 - 1.2	0.8 - 1.2
L5	0.25 - 0.5	0.45 - 0.65	0.5 - 0.75	0.65 - 0.95	0.65 - 0.95	0.5 - 0.8	0.5 - 0.9	0.8 - 1.2	0.6 - 1.0
L6	0.3 - 0.5	0.35 - 0.55	0.3 - 0.45	0.65 - 0.95	0.7 - 1.0	0.65 - 0.95	0.5 - 0.8	0.6 - 1.0	0.8 - 1.2

For ligand L1, at least two of the five distances shown in this table are preferably complied with.

3) Organic platform

The third step is the final step for obtaining a drug which is easily used orally. It consists in replacing the peptide platform with an organic structure in compliance with the spatial positioning of the phospholipid ligands. The calcium and phospholipid ligands are no longer amino acid residues but chemical functions reproducing the interactions described earlier.

With the organic structures currently used in pharmacology, it is possible to build stiff platforms capable of having a site for binding the phospholipid, according to the invention. These structures may be formed through conventional chemical techniques known to one skilled in the art, for which a reminder is unnecessary here.

Example 5

Very advantageously, use of a structure or assembly of the present invention may be made as indicated earlier in three directions: research, diagnose and therapeutics.

1) Research

For these experiments, it is appropriate to couple a structure of the present invention with a labelling molecule enabling a detection to be performed. These labelling molecules may be the aforementioned ones, for example the fluorescent molecules, an avidin-biotin system, radioelements and generally speaking, those currently used.

2) Diagnose

The chemical structures and assemblies of the present invention may be used, as indicated earlier, for "*in vitro*" detection of pathologies involving the occurrence of negative charges at the surface of cells and the release of microvesicles in blood: for example, coagulation disorders, acute inflammatory pathologies,

etc.

They may also be coupled with short-lived radioelements and with "*in vivo*" detection of the localization of thrombotic areas during vascular accidents of any kind, in particular cerebrovascular accidents, through the use of imaging systems.

They may also be coupled with paramagnetic compounds, for example a gadolinium complex, and with "*in vivo*" detection of the localization of thrombotic areas during vascular accidents of any kind, in particular cerebrovascular accidents, by using magnetic resonance imaging (MRI).

The aforementioned couplings may be achieved through standard organic chemistry techniques known to one skilled in the art, for which a reminder is unnecessary here.

3) The drug

The structures and assemblies of the present invention may be used as such for producing a drug which may be used for a treatment or a prophylaxis since they have intrinsic anticoagulant, antithrombolytic and anti-inflammatory properties.

With the assemblies according to the invention, a cladding of cell surfaces may be achieved, capable of blocking access of compounds involved in the primary stages of blood coagulation and inflammatory phenomena at these surfaces.

The structures and assemblies of the present invention may also be used for targeting molecules at a

site of the thrombus, of the inflammation, or towards a tumor area.

In this use, the structures and assemblies of the present invention are coupled with a molecule which has a thrombolytic action, with a molecule which has an anti-inflammatory action or with a molecule which has an anti-tumor action, respectively.

The structures and assemblies of the present invention may therefore for example be used for producing a drug which may be used in the treatment and prophylaxis of thrombosis. Coupling of these structures and assemblies to molecules with thrombolytic action allows the latter to be targeted towards the thrombogenic areas. Thrombolytic molecules such as streptokinase, urokinase and plasminogen activators may be used.

Structures and assemblies of the present invention may also be used coupled with a molecule having an anti-inflammatory action in order to produce a drug which may for example be used locally or orally in acute pathologies like asthma, HRC, Crohn's decease, septic shock, collagenosis and arthritis.

The structures and assemblies of the present invention may also be used coupled with a molecule having an anti-tumor action. This coupling enables the latter molecule to be targeted towards the areas bearing negative charges such as tumors having apoptotic cell centers, inflammatory tumors, etc.

The structures and assemblies of the present invention may also be used for producing a cover

material for biomaterials likely to be thrombogenic. A thrombogenic biomaterial covered in this way loses its thrombogenic properties. For example, the thrombogenic biomaterial may be a heart valve.

The invention provides the use of a chemical structure derived from proteins of the annexin family and their isolated, changed or unchanged domains, capable of binding reversibly to lipid effectors such as phosphatidylserines, phosphatidic acids, phosphatidylethanolamines and phosphatidylinosito-phosphates. The aim is to provide a set of protein, peptide, peptoid and organic compounds, for which the main property is specific recognition of the occurrence of lipid signals at the surface of cell membranes in relationship with the normal or pathological functioning of tissues. Pathologies especially targeted by the invention are: (i) blood coagulation disorders, (ii) apoptosis phenomena subsequent to the action of chemical compounds, physical effects like ionizing radiation, biological effects like those related to the formation or necrosis of cancerous tissues, in addition to the normal phenomena of apoptosis, (iii) acute inflammatory pathologies and (iv) disorders associated with relationships between the cells and the extracellular matrix and notably with collagen.

In addition to the complete engineering of entire annexins, one of the aspects of the invention is the use of annexin covalent modules and domains either directly or as a platform for the engineering of functional peptide compounds. The aim is to use these

domains and modules either in their natural form, or modified through mutagenetic or chemical routes, to transform them into compounds meeting the biological criteria discussed in the previous paragraph.

Because of their small size, these domains may easily be associated with other proteins either for forming multifunctional chimera proteins, or for introducing a controlling mechanism by effectors other than the signalling phospholipid. Further, the invention provides redefinition, through protein engineering methods, of the specificity of domains for the different signalling lipids mentioned above.

The invention finally provides reconstruction of these domains, through a novel design, in order to transform them into compounds with a more limited size and accessible to peptide synthesis and in particular to the introduction of non-natural amino acid residues with the purpose of increasing the lifetime of these compounds in the organism.

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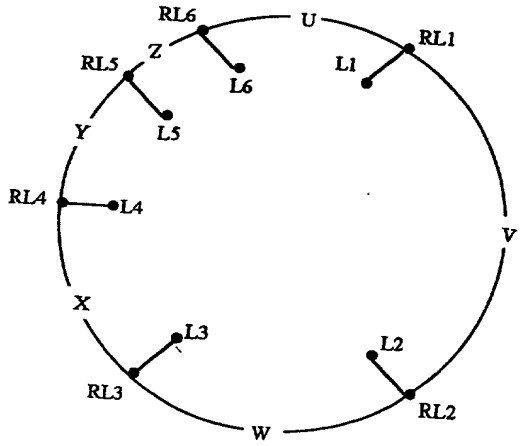
CLAIMS

1. A chemical structure with an affinity for a phospholipid, consisting of at least a chemical platform U, V, W, X, Y, Z including six residues RL1, RL2, RL3, RL4, RL5, RL6 supporting a set of chemical functions which may bind to said phospholipid, called, L1, L2, L3, L4, L5, L6 respectively, wherein these chemical functions L define the affinity of said structure for said phospholipid, said structure having one of the following constructions (I), (II) and (III):



(I)

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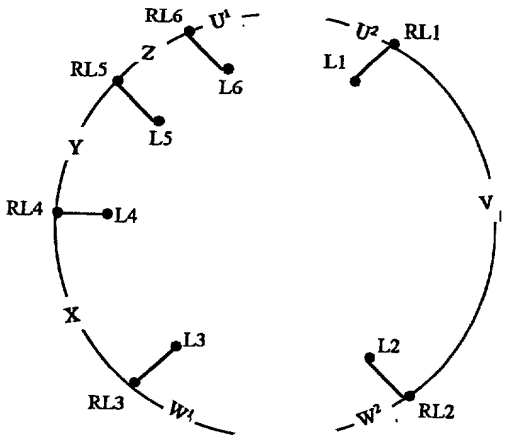
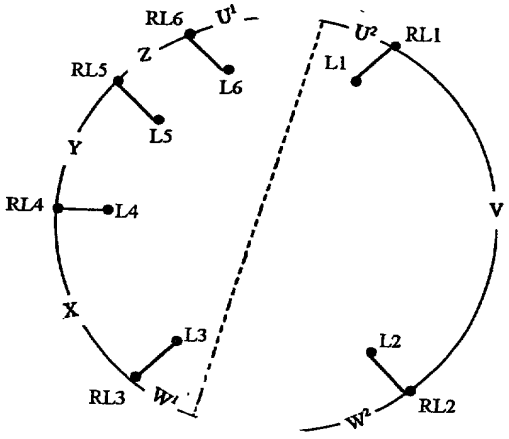


(II)



(III)

wherein U, U^1, U^2, V, W, W^1, W^2, X, Y, Z are independently a natural or non-natural amino-acid, a peptide consisting of natural or non-natural amino-acids, a carbon chain, or carbon cyclic group(s),

wherein RL1 to RL6 are selected from molecules having the binding chemical functions L1 to L6, respectively, wherein said chemical functions comprise either at least a positive charge, donor of a hydrogen bond, or at least a negative charge, acceptor of a hydrogen bond, and

wherein U, U^1, U^2, V, W, W^1, W^2, X, Y, Z are such that RL6 and RL1 are distant from 0.65 to 0.95 nm, L6 and L1 are distant from 0.65 to 0.9 nm, RL1 and RL2 are distant from 0.45 to 0.65 nm, L1 and L2 are distant from 0.4 to 0.55 nm, RL2 and RL3 are distant from 0.5 to 1.05 nm, L2 and L3 are distant from 0.4 to 0.6 nm, RL3 and RL4 are distant from 0.5 to 0.8 nm, L3 and L4 are distant from 0.35 to 0.5 nm, RL4 and RL5 are distant from 0.45 to 0.75 nm, and L4 and L5 are distant from 0.4 to 0.55 nm, RL5 and RL6 are distant from 0.4 to 1.2 nm, L5 and L6 are distant from 0.4 to 0.6 nm.

2. The chemical structure with an affinity for a phospholipid, consisting of at least a chemical platform a, a', b, b', c, d, e, f, g, h, i, j, k, l including 11 residues, LR1, LR2, LR3, LR4, LR5, RL6, RCa1, RCa2, RCa3, RCa4 and RCa5 supporting a set of chemical functions which may bind to said phospholipid called L1, L2, L3, L4, L5, L6, respectively, and a set of chemical functions binding to a calcium atom called LCa1, LCa2, LCa3, LCa4, LCa5, respectively, wherein these chemical functions RL1 to RCa5 define the affinity of said structure for said phospholipid, said structure having one of the following constructions (IV), (V) and (VI):

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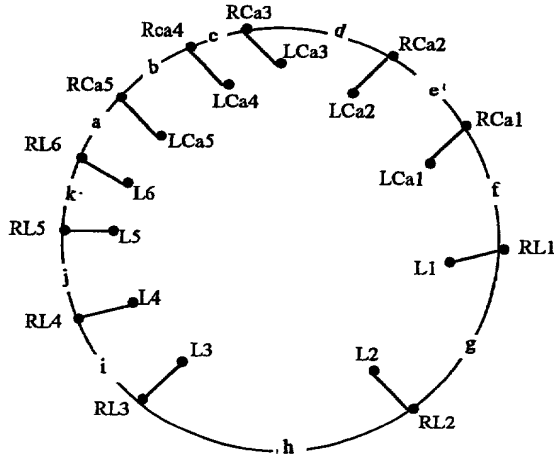
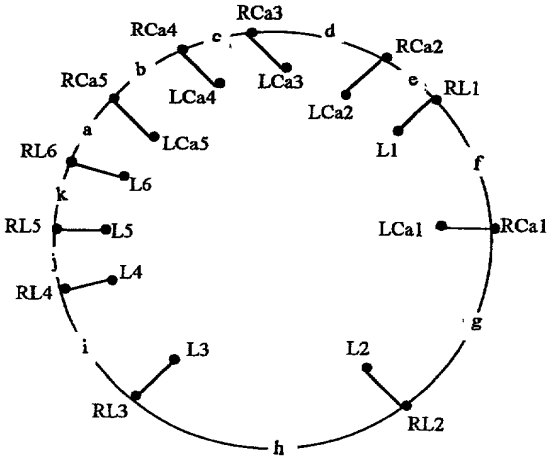


(IV)



(V)

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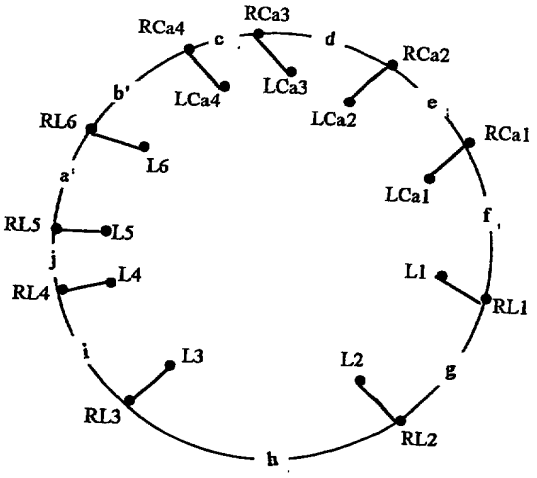

(VI)

wherein a, a', b, b', c, d, e, f, g, h, i, j, k, l, are independently a natural or non-natural amino acid, a peptide consisting of natural or non-natural amino acids, a carbon chain, or carbon cyclic group(s),

wherein RL1 to RL6 and RCa1 to RCa5 are selected from molecules having chemical binding functions L1 to L6 and LCa1 to LCa5, respectively, wherein said chemical functions L1 to L6 comprise either at least a positively charged donor of a hydrogen bond, or at least a negatively charged acceptor of a hydrogen bond, said chemical functions LCa1 to LCa5 comprising an oxygen atom, and

wherein a in the structures of construction (IV) and (V) is such that RL6 and RCa5 are distant from 0 to 0.35 nm and such that L6 and LCa5 are distant from 0 to 0.3 nm, b in the structures of construction (IV) and (V) is such that RCa5 and RCa4 are distant from 0 to 0.35 nm and such that LCa5 and LCa4 are distant from 0.2 to 0.3 nm, b' in the structure of construction (VI) is such that RL6 and RCa4 are distant from 0

to 0.35 nm and such that L6 and LCa4 are distant from 0 to 0.35 nm, c and d are such that RCa4 and RCa3 are distant from 0.5 to 0.9 nm, LCa4 and LCa3 are distant from 0.2 to 0.4 nm, RCa3 and RCa2 are distant from 0.35 to 0.6 nm, and LCa3 and LCa2 are distant from 0.22 to 0.3 nm, e, f, g, in the structures of construction (IV), (V), (VI) are such that RL1 and RL2 are distant from 0.45 to 0.65 nm, RCa1 to RCa2 are distant from 0.4 to 0.55 nm, L1 and L2 are distant from 0.4 to 0.55 nm and LCa1 and LCa2 are distant from 0.3 to 0.4 nm, h, i, j and k are such that RL2 and RL3 are distant from 0.5 to 1.05 nm, L2 and L3 are distant from 0.4 to 0.6 nm, RL3 and RL4 are distant from 0.5 to 0.8 nm, L3 and L4 are distant from 0.35 to 0.5 nm, RL4 and RL5 are distant from 0.45 to 0.75 nm, L4 and L5 are distant from 0.4 to 0.55 nm, RL5 and RL6 are distant from 0.4 to 1.2 nm, and L5 and L6 are distant from 0.4 to 0.6 nm, a' in the structure of construction (VI) is such that RL5 and RL6 are distant from 0.4 to 1.2 nm and such that L5 and L6 are distant from 0.4 to 0.6 nm, and b' in the structure of construction (VI) is such that RL6 and RCa4 are distant from 0 to 0.35 nm and such that L6 and LCa4 are distant from 0 to 0.35 nm, wherein the structure may either be closed or open at a and/or at h.

3. The chemical structure according to claim 1, wherein L1, L2, L3 and L6 each have at least a positively charged donor of a hydrogen bond, and L4 and L5 each have at least a negatively charged acceptor of a hydrogen bond.

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4. The chemical structure according to claim 1, wherein U, V, W, X, Y and Z are peptides consisting of natural and non-natural amino acids, and RL1 to RL6 are amino acids selected from a set comprising Lys, Arg, Orn, Ser, Thr, Asp and Glu, or analogs of the latter, L1 to L6 are the charge-bearing functions of the side chains of said amino acids.

5. The chemical structure according to claim 1, wherein RL1, RL2, RL3 and RL6 are independently selected from Arg, Lys, Orn,

wherein RL4 is independently selected from Asp or Glu, and

wherein RL5 is independently selected from Ser, Thr, Asp or Glu, wherein the side chains of these amino acids have chemical functions for binding to the phospholipids L1 to L6, respectively.

6. The chemical structure according to claim 3, wherein the chemical binding functions L1 to L6 are directly accessible to the negatively charged phospholipid.

7. The chemical structure according to claim 1, further comprising a calcium site where the calcium ion complexed by this site is one of the ligands of the phospholipid.

8. The chemical structure according to claim 2, wherein a or a', b or b', c, d, e, f, g, h, i, j, k are peptides consisting of natural or non-natural amino

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acids, and RL1 to RL6 are amino acids selected from a set comprising Lys, Arg, Orn, Ser, Thr, Asp and Glu, or analogs of the latter, L1 to L6 and LCa1 to LCa5 are the charge-bearing functions of the side chains of said amino acids, and RCa1 to RCa5 are natural or non-natural amino acids.

9. The chemical structure according to claim 8, wherein the chemical binding functions L1 to L6 and the positive charges of the calcium atom when it is bound to the binding functions LCa1 to LCa5, are directly accessible to the phospholipid.

10. The chemical structure according to any of claims 1 to 9, wherein the platform is a portion of a domain of the annexin or of a modified domain of the annexin, comprising at least said residual ligands, RL1 to RL6, having said functions L1 to L6 for binding to the phospholipid respectively.

11. The chemical structure according to claim 10, wherein the annexin domain is selected from the domain 1 of annexin V shown in Fig. 6b, domain 2 of annexin I shown in Fig. 6a, domain 2 of annexin III shown in Fig. 6c and domain 1 and 2 of annexin IV shown in Fig. 6d.

12. The chemical structure according to claim 11, wherein the residual ligands RL1 to RL6 respectively are either the residues Arg25, Lys29, Arg63, Asp68, Ser71 and Glu72 of domain 1 of annexin V shown in

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Fig. 6b or residues Arg124, Lys128, Arg162, Asp167, Ser170 and Asp171 of domain 2 of annexin I shown in Fig. 6a, or residues Lys100, Lys104, Lys138, Asp143, Ser146 and Glu147 of domain 2 of annexin III shown in Fig. 6c, or residues Arg96, Lys101, Arg135, Asp140, Ser143 and Asp144 of domain 2 of annexin IV shown in Fig. 6d, or residues Arg24, Lys28, Arg62, Asp67, Ser70 and Glu71 of domain 1 of annexin IV shown in Fig. 6d.

13. A chemical structure with an affinity for a phospholipid, characterized in that it comprises a molecule with the following formula (VII):

$$RL1\text{-}N^1\text{-}RL2\text{-}M\text{-}RL3\text{-}N^2\text{-}RL4\text{-}N^3\text{-}RL5\text{-}RL6 \quad \text{(VII)}$$

wherein N^1 to N^3 each independently represent 1 to 4, independently selected, natural or non-natural, amino acids and wherein M is a peptide consisting of 1 to 100 natural or non-natural amino acids

wherein RL1, RL2, RL3 and RL6 are independently selected from Lys, Arg or Orn; RL4 is independently selected from Asp or Glu; and RL5 is independently selected from Ser, Thr, Asp, or Glu, wherein said structure is linear or cyclic.

14. The chemical structure according to claim 13, wherein N^1 represents three amino acids, N^2 represents four amino acids, and N^3 represents two amino acids.

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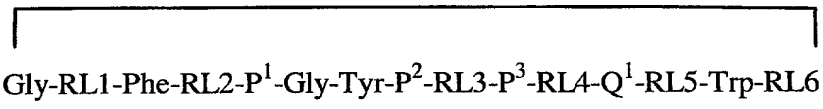
15. The chemical structure according to claim 13 or 14, wherein M is a peptide consisting of 33 natural or non-natural amino acids.

16. The chemical structure according to claim 13, wherein the structure of formula (VII) is a peptide sequence selected from the peptide sequence from Arg124 to Asp171 in the ID No.1 sequence shown in Fig. 6a, the peptide sequence from Arg25 to Glu72 in the ID No.2 sequence shown in Fig. 6b, the peptide sequence from Lys100 to Glu147 in the ID No.3 sequence shown in Fig. 6c, the sequence from Arg24 to Glu71 in the ID No.4 sequence shown in Fig. 6d, the sequence from Arg96 to Asp144 in ID No.5 sequence shown in Fig. 6, or a modified sequence of these sequences provided that RL1, RL2, RL3 and RL6 are independently selected from Lys, Arg, or Orn, RL4 is independently selected from Asp or Glu, and RL5 is independently selected from Ser, Thr, Asp or Glu.

17. A chemical structure with an affinity for a phospholipid, characterized in that it comprises at least a portion of a peptide sequence selected from ID No.1 sequence shown in Fig. 6a, ID No.2 sequence shown in Fig. 6b, ID No.3 sequence shown in Fig. 6c, and ID No.4 and No.5 sequences shown in Fig. 6d or a modified sequence of the latter.

18. A chemical structure with an affinity for a negatively charged phospholipid, characterized in that

it comprises a cyclic peptide sequence of the following formula (VIII):



Gly-RL1-Phe-RL2-P^1-Gly-Tyr-P^2-RL3-P^3-RL4-Q^1-RL5-Trp-RL6

wherein RL1 and RL6 are independently selected from Lys, Orn and Arg; RL2 and RL3 are Arg; RL4 and RL5 are independently selected from Asp and Glu;

wherein P^1, P^2 and P^3 are independently selected from Ser and Thr; wherein Q^1 is selected from Gly and Met.

19. The chemical structure according to any of claims 13 to 17, further comprising a calcium site where the calcium ion is complexed by this site forms one of the ligands of the negatively charged phospholipid.

20. The chemical structure according to any of the preceding claims, said structures having an affinity for a phospholipid selected from a phosphatidylserine, a phosphatidylethanolamine, a phosphatidylinositol, a phosphatidic acid, and a cardiolipid.

21. A chemical assembly having an affinity for a phospholipid, characterized in that it comprises at least two identical or different chemical structures defined in claims 1 to 20, said structures being bound.

22. A chemical assembly according to claim 23,

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wherein at least one of the chemical structures is one of the chemical structures defined in claims 13 to 20.

23. A method for producing a chemical structure as defined in any of the preceding claims 10 to 20, characterized in that it comprises steps consisting of preparing a cDNA comprising a coding sequence of bases for said chemical structure, inserting the cDNA in an appropriate expression vector, transforming an appropriate host cell for replicating the plasmid and producing said structure by translation of said cDNA.

24. The method according to claim 23, wherein the vector is a plasmid.

25. The method according to claim 23, wherein the vector is a pGEX-2T vector.

26. The method according to claim 23, 24 or 25 wherein the appropriate host cell is *E. Coli*.

27. A use of a chemical structure as defined in claims 1 to 20 for preparing a drug.

28. A use of a chemical assembly as defined in claims 21 or 22 for preparing a drug.

29. The use according to claim 27 or 28, wherein the drug is selected from a drug for treating a thrombosis, a drug for treating a tumor, a drug with an anti-inflammatory action.

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30. A use of a structure as defined in claims 1 to 19 for producing a material for covering thrombogenic biomaterial.

31. A labelling compound characterized in that it comprises a structure as defined in claims 1 to 20 coupled with a labelling molecule.

32. A labelling compound characterized in that it comprises an assembly as defined in claim 21 or 22 coupled with a labelling molecule.

33. The compound according to claim 31 or 32, wherein the labelling molecule is selected from a fluorescent molecule, the avidin-biotin complex, a radioelement, and a paramagnetic compound.

34. A diagnose kit comprising a compound according to any of claims 31 to 32.

35. The diagnose kit according to claim 34, further comprising an adequate reagent enabling said labelling molecule to be detected.

36. A kit for analyzing and detecting negative charges at the surface of cells, characterized in that it comprises a structure according to any of claims 1 to 20, coupled with a tracer.

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37. A kit for analyzing and detecting negative charges at the surface of cells, characterized in that it comprises an assembly according to any of claims 21 or 22, coupled with a tracer.

38. A kit for analyzing and detecting microvesicles in blood at the surface of cells, characterized in that it comprises a structure according to any of claims 1 to 20, coupled with a tracer.

39. A kit for analyzing and detecting microvesicles in blood at the surface of cells, characterized in that it comprises an assembly according to any of claims 21 to 22, coupled with a tracer.

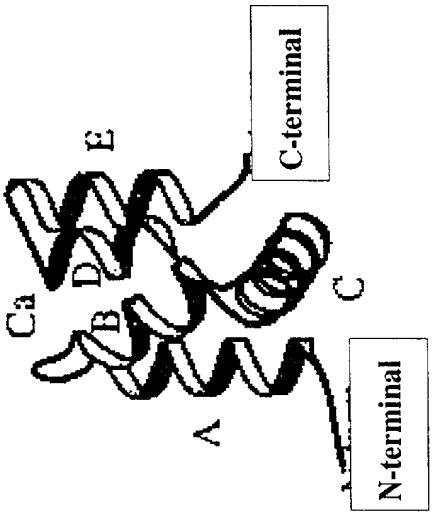

FIG. 1B

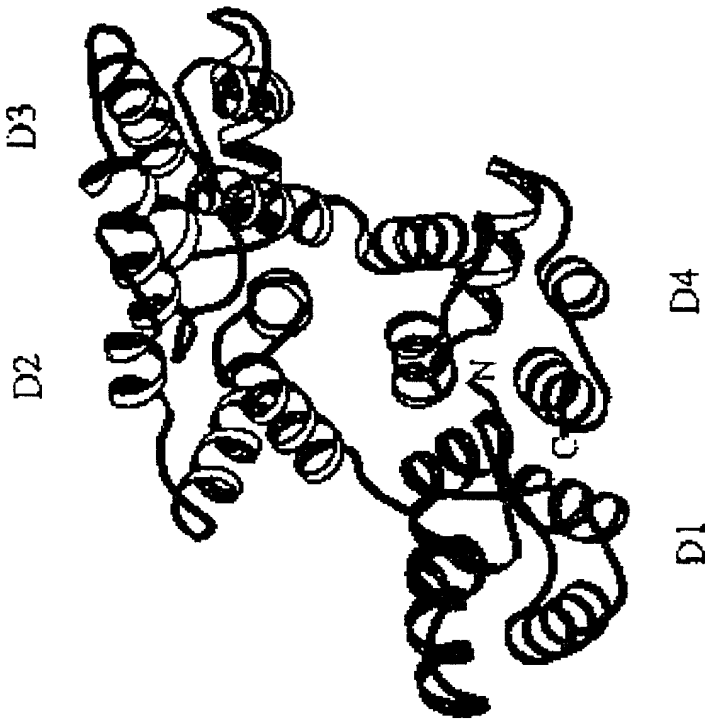

FIG. 1A

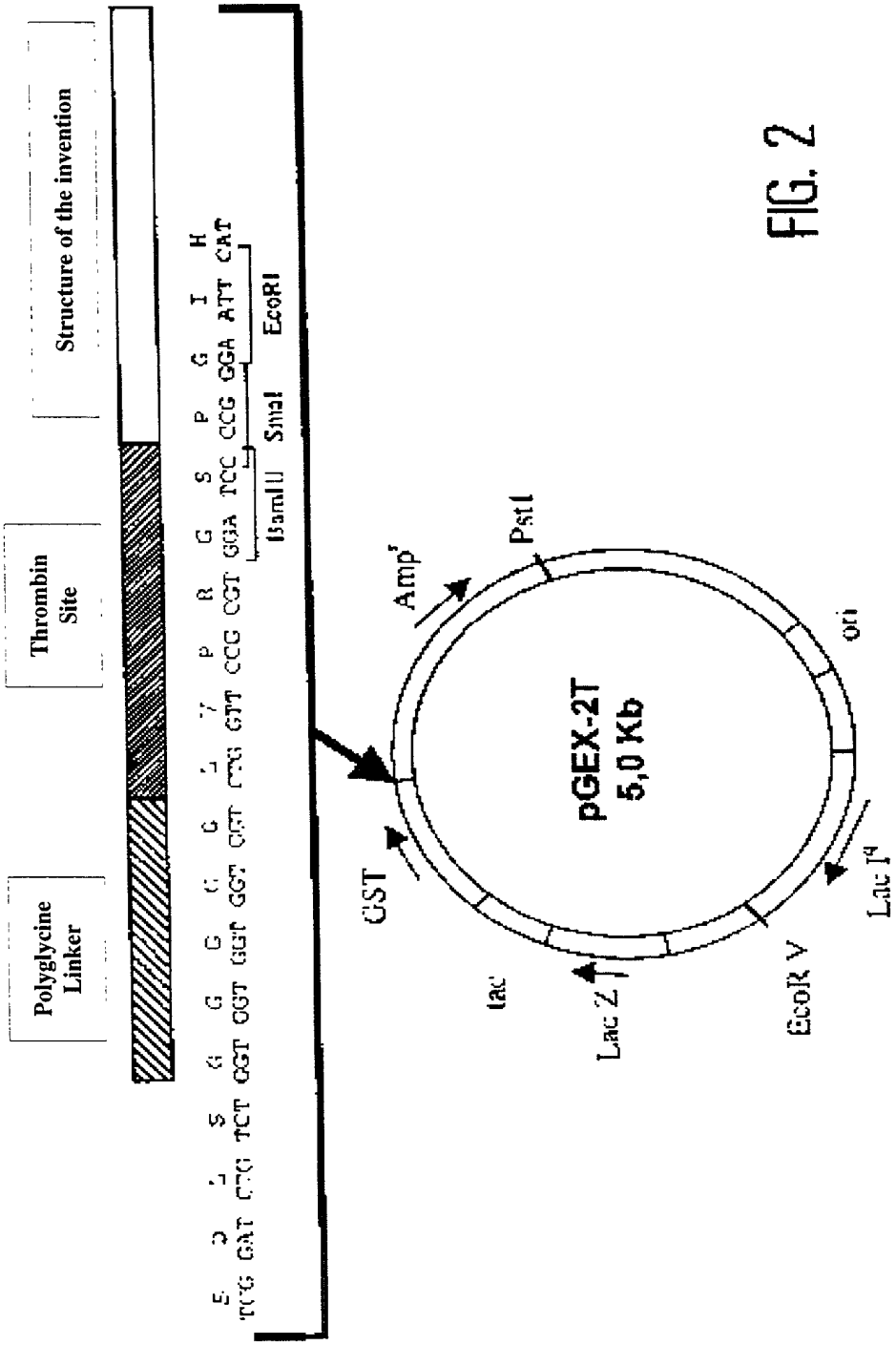
Polyglycine Linker
Thrombin Site
Structure of the invention
BamHI SmaI EcoRI
pGEX-2T
5,0 Kb
Amp'
PstI
ori
GST
lac
Lac Z
EcoR V
Lac I^q

FIG. 2

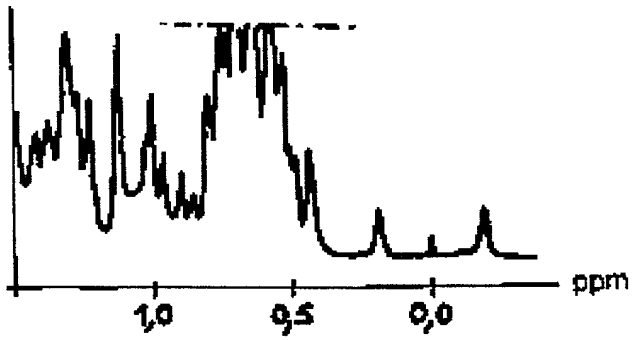

FIG. 3

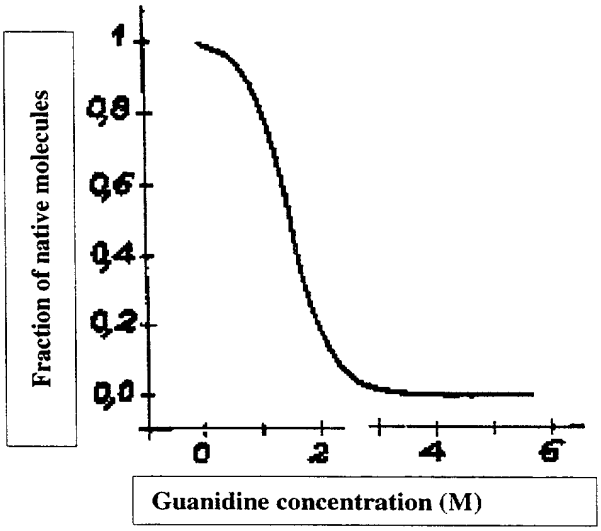

FIG. 4

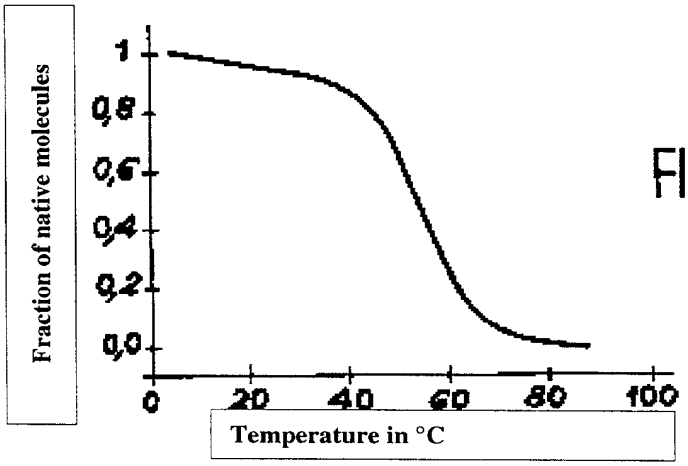

FIG. 5

Sequence ID No. 1

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Domain 2

```
Met Ala Met Val Ser Glu Phe Leu Lys Gln Ala Trp Phe Ile
1               5                  10
Glu Asn Glu Glu Gln Glu Tyr Val Gln Thr Val Lys Ser Ser
15                  20                  25
Lys Gly Gly Pro Gly Ser Ala Val Ser Pro Tyr Pro Thr Phe
     30                  35                  40
Asn Pro Ser Ser Asp Val Ala Ala Leu His Lys Ala Ile Met
         45                  50                  55
Val Lys Gly Val Asp Glu Ala Thr Ile Ile Asp Ile Leu Thr
             60                  65                  70
Lys Arg Asn Asn Ala Gln Arg Gln Gln Ile Lys Ala Ala Tyr
         75                  80
Leu Gln Glu Thr Gly Lys Pro Leu Asp Glu Thr Leu Lys Lys
85                  90                  95
Ala Leu Thr Gly His Leu Glu Glu Val Val Leu Ala Leu Leu
    100                 105                 110
Lys Thr Pro Ala Gln Phe Asp Ala Asp Glu Leu Arg Ala Ala
        115                 120                 125
Met Lys Gly Leu Gly Thr Asp Glu Asp Thr Leu Ile Glu Ile
            130                 135                 140
Leu Ala Ser Arg Thr Asn Lys Glu Ile Arg Asp Ile Asn Arg
        145                 150
Val Tyr Arg Glu Glu Leu Lys Arg Asp Leu Ala Lys Asp Ile
155                 160                 165
Thr Ser Asp Thr Ser Gly Asp Phe Arg Asn Ala Leu Leu Ser
    170                 175                 180
Leu Ala Lys Gly Asp Arg Ser Glu Asp Phe Gly Val Asn Glu
        185                 190                 200
Asp Leu Ala Asp Ser Asp Ala Arg Ala Leu Tyr Glu Ala Gly
            205                 210                 215
Glu Arg Arg Lys Gly Thr Asp Val Asn Val Phe Asn Thr Ile
        220                 225
Leu Thr Thr Arg Ser Tyr Pro Gln Leu Arg Arg Val Phe Gln
230                 235                 240
Lys Tyr Thr Lys Tyr Ser Lys His Asp Met Asn Lys Val Leu
    245                 250                 260
Asp Leu Glu Leu Lys Gly Asp Ile Glu Lys Cys Leu Thr Ala
        265                 270                 275
Ile Val Lys Cys Ala Thr Ser Lys Pro Ala Phe Phe Ala Glu
            280                 285                 290
Lys Leu His Gln Ala Met Lys Gly Val Gly Thr Arg His Lys
        295                 300
Ala Leu Ile Arg Ile Met Val Ser Arg Ser Glu Ile Asp Met
305                 310                 315
Asn Asp Ile Lys Ala Phe Tyr Gln Lys Met Tyr Gly Ile Ser
    320                 325                 330
Leu Cys Gln Ala Ile Leu Asp Glu Thr Lys Gly Asp Tyr Glu
        335                 340                 345
Lys Ile Leu Val Ala Leu Cys Gly Gly Asn
            350                 355
```

FIG. 6A: Human annexin I

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Sequence ID No. 2

Domain 1

```
Met Ala Gln Val Leu Arg Gly Thr Val Thr Asp Phe Pro Gly
1               5                  10
Phe Asp Glu Arg Ala Asp Ala Glu Thr Leu Arg Lys Ala Met
15                  20                  25
Lys Gly Leu Gly Thr Asp Glu Glu Ser Ile Leu Thr Leu Leu
            30                  35                  40
Thr Ser Arg Ser Asn Ala Gln Arg Gln Glu Ile Ser Ala Ala
        45                  50                  55
Phe Lys Thr Leu Phe Gly Arg Asp Leu Leu Asp Asp Leu Lys
    60                  65                  70
Ser Glu Leu Thr Gly Lys Phe Glu Lys Leu Ile Val Ala Leu
75                  80
Met Lys Pro Ser Arg Leu Tyr Asp Ala Tyr Glu Leu Lys His
            85                  90
Ala Leu Lys Gly Ala Gly Thr Asn Glu Lys Val Leu Thr Glu
        95                  100                 105
Ile Ile Ala Ser Arg Thr Pro Glu Glu Leu Arg Ala Ile Lys
    110                 115                 120
Gln Val Tyr Glu Glu Glu Tyr Gly Ser Ser Leu Glu Asp Asp
125                 130                 135
Val Val Gly Asp Thr Ser Gly Tyr Tyr Gln Arg Met Leu Val
            140                 145
Val Leu Leu Gln Ala Asn Arg Asp Pro Asp Ala Gly Ile Asp
150                 155                 160
Glu Ala Gln Val Glu Gln Asp Ala Gln Ala Leu Phe Gln Ala
        165                 170                 175
Gly Glu Leu Lys Trp Gly Thr Asp Glu Glu Lys Phe Ile Thr
    180                 185                 190
Ile Phe Gly Thr Arg Ser Val Ser His Leu Arg Lys Val Phe
195                 200                 205
Asp Lys Tyr Met Thr Ile Ser Gly Phe Gln Ile Glu Glu Thr
            210                 215
Ile Asp Arg Glu Thr Ser Gly Asn Leu Glu Gln Leu Leu Leu
220                 225                 230
Ala Val Val Lys Ser Ile Arg Ser Ile Pro Ala Tyr Leu Ala
        235                 240                 245
Glu Thr Leu Tyr Tyr Ala Met Lys Gly Ala Gly Thr Asp Asp
    250                 255                 260
His Thr Leu Ile Arg Val Met Val Ser Arg Ser Glu Ile Asp
265                 270                 275
Leu Phe Asn Ile Arg Lys Glu Phe Arg Lys Asn Phe Ala Thr
            280                 285
Ser Leu Tyr Ser Met Ile Lys Gly Asp Thr Ser Gly Asp Tyr
290                 295                 300
Lys Lys Ala Leu Leu Leu Leu Cys Gly Glu Asp Asp
305                 310                 315
```

FIG. 6B: **Human annexin V**

Sequence ID No. 3

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```
Met Ala Ser Ile Trp Val Gly His Arg Gly Thr Val Arg Asp
1               5                  10
Tyr Pro Asp Phe Ser Pro Ser Val Asp Ala Glu Ala Ile Gln
15                  20                  25
Lys Ala Ile Arg Gly Ile Gly Thr Asp Glu Lys Met Leu Ile
        30                  35                  40
Ser Ile Leu Thr Glu Arg Ser Asn Ala Gln Arg Gln Leu Ile
            45                  50                  55
Val Lys Glu Tyr Gln Ala Ala Tyr Gly Lys Glu Leu Lys Asp
        60                  65                  70
Asp Leu Lys Gly Asp Leu Ser Gly His Phe Glu His Leu Met
    75                  80
Val Ala Leu Val Thr Pro Pro Ala Val Phe Asp Ala Lys Gln
85                  90                  95
Leu Lys Lys Ser Met Lys Gly Ala Gly Thr Asn Glu Asp Ala
        100                 105                 110
Leu Ile Glu Ile Leu Thr Thr Arg Thr Ser Arg Gln Met Lys
    115                 120                 125
Asp Ile Ser Gln Ala Tyr Tyr Thr Val Tyr Lys Lys Ser Leu
130                 135                 140
Gly Asp Asp Ile Ser Ser Glu Thr Ser Gly Asp Phe Arg Lys
145                 150
Ala Leu Leu Thr Leu Ala Asp Gly Arg Arg Asp Glu Ser Leu
155                 160                 165
Lys Val Asp Glu His Leu Ala Lys Gln Asp Ala Gln Ile Leu
        170                 175                 180
Tyr Lys Ala Gly Glu Asn Arg Trp Gly Thr Asp Glu Asp Lys
    185                 190                 195
Phe Thr Glu Ile Leu Cys Leu Arg Ser Phe Pro Gln Leu Lys
200                 205                 210
Leu Thr Phe Asp Glu Tyr Arg Asn Ile Ser Gln Lys Asp Ile
215                 220
Val Asp Ser Ile Lys Gly Glu Leu Ser Gly His Phe Glu Asp
225                 230                 235
Leu Leu Leu Ala Ile Val Asn Cys Val Arg Asn Thr Pro Ala
        240                 245                 250
Phe Leu Ala Glu Arg Leu His Arg Ala Leu Lys Gly Ile Gly
    255                 260                 270
Thr Asp Glu Phe Thr Leu Asn Arg Ile Met Val Ser Arg Ser
270                 280                 285
Glu Ile Asp Leu Leu Asp Ile Arg Thr Glu Phe Lys Lys His
290                 295
Tyr Gly Tyr Ser Leu Tyr Ser Ala Ile Lys Ser Asp Thr Ser
300                 305                 310
Gly Asp Tyr Glu Ile Thr Leu Leu Lys Ile Cys Gly Gly Asp Asp
    315                 320                 325
```

Domain 2

FIG. 6C: Human annexin III

Sequence ID No. 4

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Domain 1

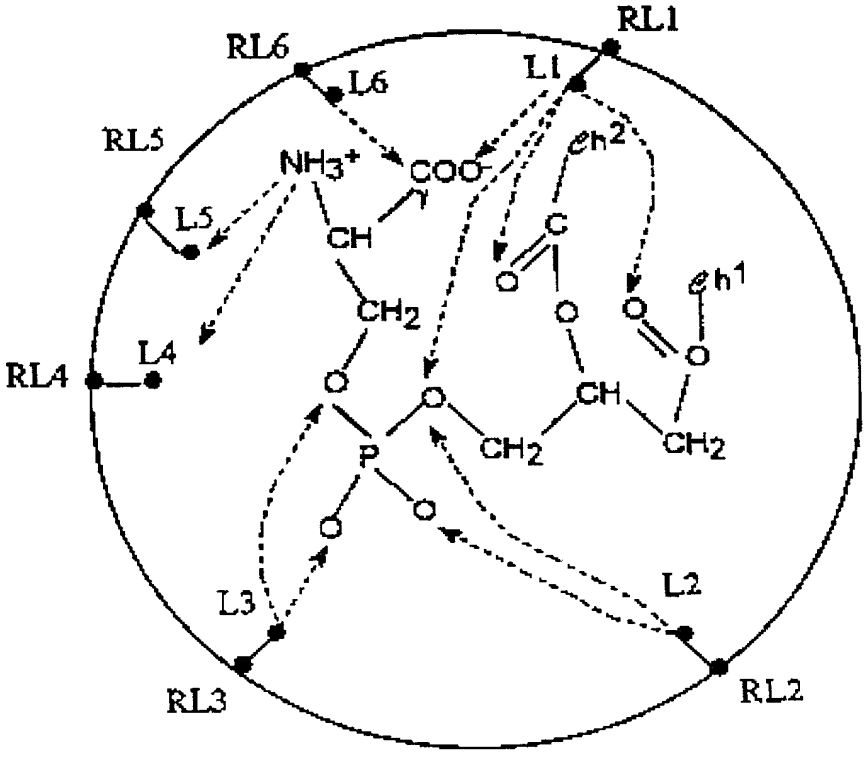
```
Met Ala Thr Lys Gly Gly Thr Val Lys Ala Ala Ser Gly Phe
1               5                  10
Asn Ala Met Glu Asp Ala Gln Thr Leu Arg Lys Ala Met Lys
15                 20                  25
Gly Leu Gly Thr Asp Glu Asp Ala Ile Ile Ser Val Leu Ala
            30                  35                  40
Tyr Arg Asn Thr Ala Gln Arg Gln Glu Ile Arg Thr Ala Tyr
        45                  50                  55
Lys Ser Thr Ile Gly Arg Asp Leu Ile Asp Asp Leu Lys Ser
                60                  65                  70
Glu Leu Ser Gly Asn Phe Glu Gln Val Ile Val Gly Met Met
            75                  80
Thr
85
```

Sequence ID No. 5

Domain 2

```
Pro Thr Val Leu Tyr Asp Val Gln Glu Leu Gln Arg Lys
86                  90                  95
Ala Met Lys Gly Ala Gly Thr Asp Glu Gly Cys Leu Ile Glu
    100                 105                 110
Ile Leu Ala Ser Arg Thr Pro Glu Glu Ile Arg Arg Ile Asn
        115                 120                 125
Gln Thr Tyr Gln Leu Gln Tyr Gly Arg Ser Leu Glu Asp Asp
    130                 135                 140
Ile Arg Ser Asp Thr Ser Phe Met Phe Gln Arg Val Leu Val
145                 150
Ser Leu Ser Ala Gly Gly Arg Asp Glu Gly Asn Tyr Leu Asp
155                 160                 170
Asp Ala Leu Val Arg Gln Asp Ala Gln Asp Leu Tyr Glu Ala
    175                 180                 185
Gly Glu Lys Lys Trp Gly Thr Asp Glu Val Lys Phe Leu Thr
    190                 195                 200
Val Leu Cys Ser Arg Asn Arg Asn His Leu Leu His Val Phe
        205                 210                 215
Asp Glu Tyr Lys Arg Ile Ser Gln Lys Asp Ile Glu Gln Ser
    220                 225
Ile Lys Ser Glu Thr Ser Gly Ser Phe Glu Asp Ala Leu Leu
230                 235                 240
Ala Ile Val Lys Cys Met Arg Asn Lys Ser Ala Tyr Phe Ala
    245                 250                 255
Glu Lys Leu Tyr Lys Ser Met Lys Gly Leu Gly Thr Asp Asp
    260                 265                 270
Asn Thr Leu Ile Arg Val Met Val Ser Arg Ala Glu Ile Asp
        275                 280                 285
Met Leu Asp Ile Arg Ala His Phe Lys Arg Leu Tyr Gly Lys
    290                 295
Ser Leu Tyr Ser Phe Ile Lys Gly Asp Thr Ser Gly Asp Tyr
300                 305                 310
Arg Lys Val Leu Leu Val Leu Cys Gly Gly Asp Asp
    315                 320                 325
```

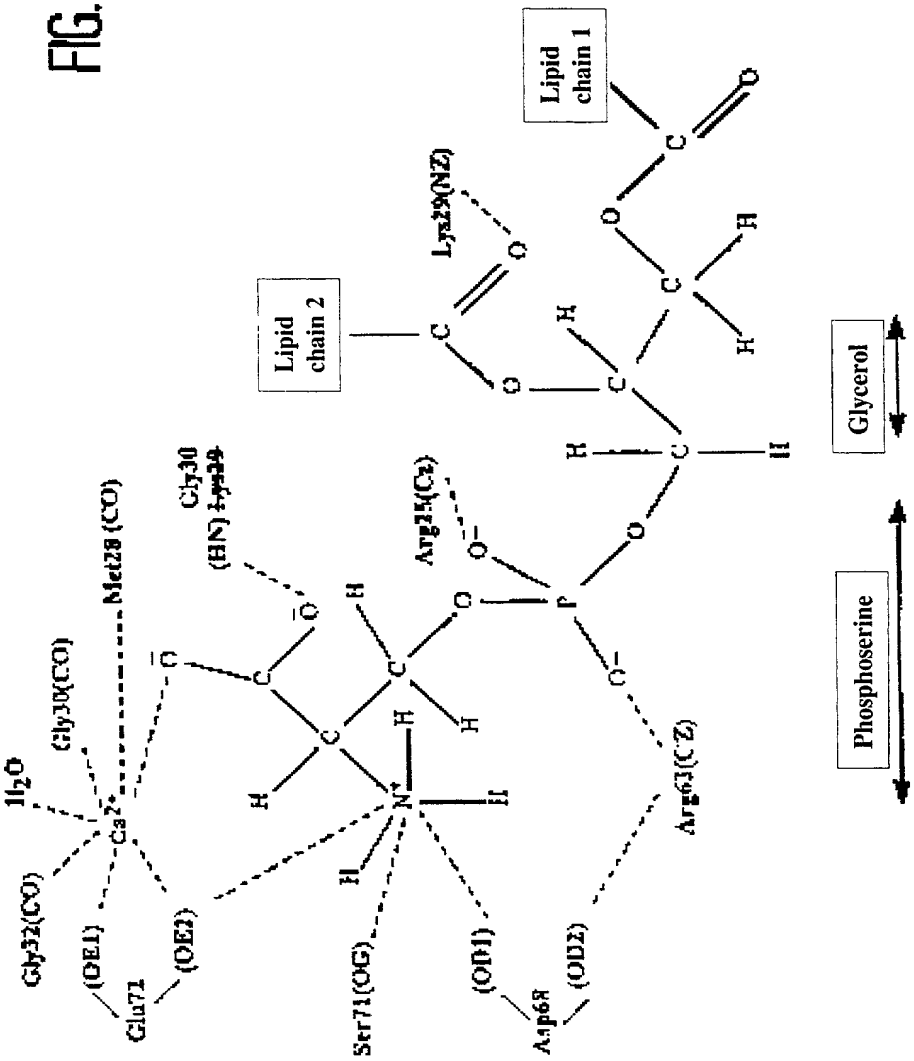
FIG. 6D: **Human annexin IV**



Compound (I) + phosphatidylserine

FIG. 7

FIG. 8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Lipid chain 2
Lys29(NZ)
Glycerol
Phosphoserine
Arg54(Cz)
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Met28 (CO)
Gly30(CO)
Gly32(CO)
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Glu71
(OE2)
Ser71(OG)
(OD1)
Asp68
(OD2)
Arg63(CZ)

10/10

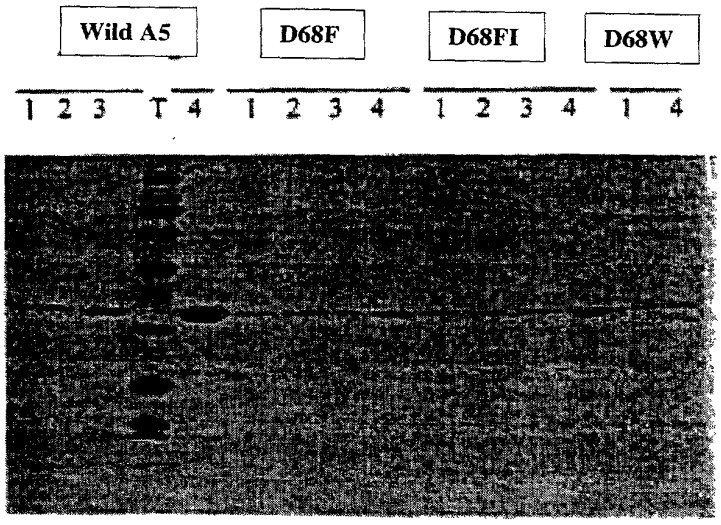

FIG. 9 A

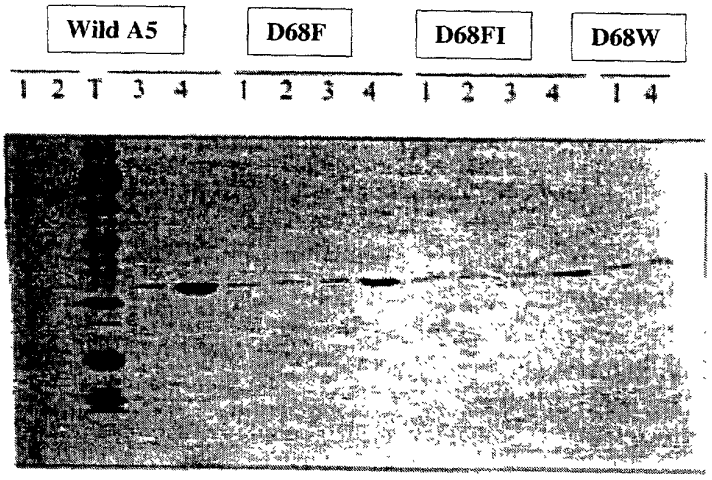

FIG. 9 B

B 13117.3 EE

Declaration, Power Of Attorney and Petition

WE (I) the undersigned inventor(s), hereby declare(s) that :

My residence, post office address and citizenship are as stated below next to my name,

We (I) believe that we are (I am) the original, first, and joint (sole) inventor(s) of the subject matter which is claimed and for which a patent is sought on the invention entitled
CHEMICAL STRUCTURE HAVING AN AFFINITY FOR A PHOSPHOLIPID AND LABELLING COMPOUND , DIAGNOSE KIT, AND DRUG COMPRISING THIS STRUCTURE

the specification of which

- ☐ is attached hereto.
- ☐ was filed on
  as Application Serial No.
  and amended on
- ☒ was filed as PCT international application
  Number PCT/FR99/02329
  on September 30, 1999
  and was amended under PCT Article 19
  on November 27, 2000

We (I) hereby state that we (I) have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

We (I) acknowledge the duty to disclose information known to be material to the patentability of this application as defined in Section 1.56 of Title 37 Code of Federal Regulations.

We (I) hereby claim foreign priority benefits under 35 U.S.C. § 119 (a)-(d) or § 365 (b) of any foreign application(s) for patent or inventor's certificate, or § 365 (a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed. Prior Foreign Application (s)

Application No.	Country	Day/month/Year	Priority Claimed
98 12366	FRANCE	02 October 1998	☒ YES ☐ NO
			☐ YES ☐ NO
			☐ YES ☐ NO
			☐ YES ☐ NO

We (I) hereby claim the benefit under Title 35, United States Code, § 119 (e) of any United States provisional application(s) listed below.

(Application Number) (Filing Date)

(Application Number) (Filing Date)

We (I) hereby claim the benefit under 35 U.S.C. §120 of any United States application(s), or § 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR § 1.56 which became available between the filing date of prior application and the national or PCT International filing date of this application.

Application Serial No.	Filing Date	Status (pending, patented, abandoned)

And we (I) hereby appoint : Norman F. Oblon, Registration Number 24,618; Marvin J. Spivak, Registration Number 24,913; C, Irvin McClelland, Registration Number 21,124; Gregory J. Maier, Registration Number 25,599; Arthur I. Neustadt, Registration Number 24,854; Richard D. Kelly, Registration Number 27,757; James D. Hamilton, Registration Number 28,421; Eckhard H. Kuesters, Registration Number 28,870; Robert T. Pous, Registration Number 29,099; Charles L. Gholz, Registration Number 26,395; William E. Beaumont, Registration Number 30,996; Jean-Paul Lavalleye, Registration Number 31,451; Stephen G. Baxter, Registration Number 32,884; Richard L. Treanor, Registration Number 36,379; Steven P. Weihrouch, Registration Number 32,829; John T. Goolkasian, Registration Number 26,142; Richard L. Chinn, Registration Number 34,305; Steven E. Lipman, Registration Number 30,011; Carl E. Schlier, Registration Number 34,426; James J. Kulbaski, Registration Number 34,648; Richard A. Neifeld, Registration Number 35,299; J. Derek Mason, Registration Number 35,270; Surinder Sachar, Registration Number 34,423; Christina M. Gadiano, Registration Number 37,628; Jeffrey B. McIntyre, Registration Number 36,867; William T. Enos, Registration Number 33,128; Michael E. McKabe Jr., Registration Number 37,182, Bradley D. Lytle, Registration Number 40,073 and Michael R. Casey Registration Number 40,294 ; our (my) attorneys, with full powers of substitution and revocation, to prosecute this application and to transact all business in the Patent Office connected therewith; and we (I) hereby request that all correspondence regarding this application be sent to the firm of OBLON, SPIVAK, McCLELLAND, MAIER & NEUSTADT, P.C., whose post Office Address is : Fourth Floor, 1755 Jefferson Davis Highway, Arlington, Virginia 22202.

We (I) declare that all statements made herein of our (my) own knowledge are true and that all statements made on information and belief are believed to be true ; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such wilful false statements may jeopardise the validity of the application or any patent issuing thereon.

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Signature of Inventor

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Date

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Citizen of : FRANCE

Post Office Address : The same as residence

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NAME OF SECOND INVENTOR

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Signature of Inventor

April 12, 2001

Date

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Citizen of : FRANCE

Post Office Address : The same as residence

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Date

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Post Office Address : The same as residence

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Signature of Inventor

April 12, 2001

Date

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Citizen of : FRANCE

Post Office Address : The same as residence

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CORDIER-OCHSENBEIN, FRANCOISE
GUEROIS, RAPHAEL

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<141> 2001-04-02

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Glu Glu Gln Glu Tyr Val Gln Thr Val Lys Ser Ser Lys Gly Gly Pro
            20                  25                  30

Gly Ser Ala Val Ser Pro Tyr Pro Thr Phe Asn Pro Ser Ser Asp Val
        35                  40                  45

Ala Ala Leu His Lys Ala Ile Met Val Lys Gly Val Asp Glu Ala Thr
    50                  55                  60

Ile Ile Asp Ile Leu Thr Lys Arg Asn Asn Ala Gln Arg Gln Gln Ile
65                  70                  75                  80

Lys Ala Ala Tyr Leu Gln Glu Thr Gly Lys Pro Leu Asp Glu Thr Leu
                85                  90                  95
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100 105 110

Lys Thr Pro Ala Gln Phe Asp Ala Asp Glu Leu Arg Ala Ala Met Lys
115 120 125

Gly Leu Gly Thr Asp Glu Asp Thr Leu Ile Glu Ile Leu Ala Ser Arg
130 135 140

Thr Asn Lys Glu Ile Arg Asp Ile Asn Arg Val Tyr Arg Glu Glu Leu
145 150 155 160

Lys Arg Asp Leu Ala Lys Asp Ile Thr Ser Asp Thr Ser Gly Asp Phe
165 170 175

Arg Asn Ala Leu Leu Ser Leu Ala Lys Gly Asp Arg Ser Glu Asp Phe
180 185 190

Gly Val Asn Glu Asp Leu Ala Asp Ser Asp Ala Arg Ala Leu Tyr Glu
195 200 205

Ala Gly Glu Arg Arg Lys Gly Thr Asp Val Asn Val Phe Asn Thr Ile
210 215 220

Leu Thr Thr Arg Ser Tyr Pro Gln Leu Arg Arg Val Phe Gln Lys Tyr
225 230 235 240

Thr Lys Tyr Ser Lys His Asp Met Asn Lys Val Leu Asp Leu Glu Leu
245 250 255

Lys Gly Asp Ile Glu Lys Cys Leu Thr Ala Ile Val Lys Cys Ala Thr
260 265 270

Ser Lys Pro Ala Phe Phe Ala Glu Lys Leu His Gln Ala Met Lys Gly
275 280 285

Val Gly Thr Arg His Lys Ala Leu Ile Arg Ile Met Val Ser Arg Ser
290 295 300

Glu Ile Asp Met Asn Asp Ile Lys Ala Phe Tyr Gln Lys Met Tyr Gly
305 310 315 320

Ile Ser Leu Cys Gln Ala Ile Leu Asp Glu Thr Lys Gly Asp Tyr Glu

325 330 335

Lys Ile Leu Val Ala Leu Cys Gly Gly Asn
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20 25 30

Thr Asp Glu Glu Ser Ile Leu Thr Leu Leu Thr Ser Arg Ser Asn Ala
35 40 45

Gln Arg Gln Glu Ile Ser Ala Ala Phe Lys Thr Leu Phe Gly Arg Asp
50 55 60

Leu Leu Asp Asp Leu Lys Ser Glu Leu Thr Gly Lys Phe Glu Lys Leu
65 70 75 80

Ile Val Ala Leu Met Lys Pro Ser Arg Leu Tyr Asp Ala Tyr Glu Leu
85 90 95

Lys His Ala Leu Lys Gly Ala Gly Thr Asn Glu Lys Val Leu Thr Glu
100 105 110

Ile Ile Ala Ser Arg Thr Pro Glu Glu Leu Arg Ala Ile Lys Gln Val
115 120 125

Tyr Glu Glu Glu Tyr Gly Ser Ser Leu Glu Asp Asp Val Val Gly Asp
130 135 140

Thr Ser Gly Tyr Tyr Gln Arg Met Leu Val Val Leu Leu Gln Ala Asn
145 150 155 160

Arg Asp Pro Asp Ala Gly Ile Asp Glu Ala Gln Val Glu Gln Asp Ala
165 170 175

Gln Ala Leu Phe Gln Ala Gly Glu Leu Lys Trp Gly Thr Asp Glu Glu
180 185 190

Lys Phe Ile Thr Ile Phe Gly Thr Arg Ser Val Ser His Leu Arg Lys
195 200 205

Val Phe Asp Lys Tyr Met Thr Ile Ser Gly Phe Gln Ile Glu Glu Thr
210 215 220

Ile Asp Arg Glu Thr Ser Gly Asn Leu Glu Gln Leu Leu Leu Ala Val
225 230 235 240

Val Lys Ser Ile Arg Ser Ile Pro Ala Tyr Leu Ala Glu Thr Leu Tyr
245 250 255

Tyr Ala Met Lys Gly Ala Gly Thr Asp Asp His Thr Leu Ile Arg Val
260 265 270

Met Val Ser Arg Ser Glu Ile Asp Leu Phe Asn Ile Arg Lys Glu Phe
275 280 285

Arg Lys Asn Phe Ala Thr Ser Leu Tyr Ser Met Ile Lys Gly Asp Thr
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Ser Gly Asp Tyr Lys Lys Ala Leu Leu Leu Leu Cys Gly Glu Asp Asp
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20 25 30

Gly Ile Gly Thr Asp Glu Lys Met Leu Ile Ser Ile Leu Thr Glu Arg
35 40 45

Ser Asn Ala Gln Arg Gln Leu Ile Val Lys Glu Tyr Gln Ala Ala Tyr

```
    50                  55                  60

Gly Lys Glu Leu Lys Asp Asp Leu Lys Gly Asp Leu Ser Gly His Phe
65                  70                  75                  80

Glu His Leu Met Val Ala Leu Val Thr Pro Pro Ala Val Phe Asp Ala
                85                  90                  95

Lys Gln Leu Lys Lys Ser Met Lys Gly Ala Gly Thr Asn Glu Asp Ala
            100                 105                 110

Leu Ile Glu Ile Leu Thr Thr Arg Thr Ser Arg Gln Met Lys Asp Ile
        115                 120                 125

Ser Gln Ala Tyr Tyr Thr Val Tyr Lys Lys Ser Leu Gly Asp Asp Ile
    130                 135                 140

Ser Ser Glu Thr Ser Gly Asp Phe Arg Lys Ala Leu Leu Thr Leu Ala
145                 150                 155                 160

Asp Gly Arg Arg Asp Glu Ser Leu Lys Val Asp Glu His Leu Ala Lys
                165                 170                 175

Gln Asp Ala Gln Ile Leu Tyr Lys Ala Gly Glu Asn Arg Trp Gly Thr
            180                 185                 190

Asp Glu Asp Lys Phe Thr Glu Ile Leu Cys Leu Arg Ser Phe Pro Gln
        195                 200                 205

Leu Lys Leu Thr Phe Asp Glu Tyr Arg Asn Ile Ser Gln Lys Asp Ile
    210                 215                 220

Val Asp Ser Ile Lys Gly Glu Leu Ser Gly His Phe Glu Asp Leu Leu
225                 230                 235                 240

Leu Ala Ile Val Asn Cys Val Arg Asn Thr Pro Ala Phe Leu Ala Glu
                245                 250                 255

Arg Leu His Arg Ala Leu Lys Gly Ile Gly Thr Asp Glu Phe Thr Leu
            260                 265                 270

Asn Arg Ile Met Val Ser Arg Ser Glu Ile Asp Leu Leu Asp Ile Arg
        275                 280                 285
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Thr Glu Phe Lys Lys His Tyr Gly Tyr Ser Leu Tyr Ser Ala Ile Lys
290 295 300

Ser Asp Thr Ser Gly Asp Tyr Glu Ile Thr Leu Leu Lys Ile Cys Gly
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Gly Asp Asp

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20 25 30

Asp Glu Asp Ala Ile Ile Ser Val Leu Ala Tyr Arg Asn Thr Ala Gln
35 40 45

Arg Gln Glu Ile Arg Thr Ala Tyr Lys Ser Thr Ile Gly Arg Asp Leu
50 55 60

Ile Asp Asp Leu Lys Ser Glu Leu Ser Gly Asn Phe Glu Gln Val Ile
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Val Gly Met Met Thr
85

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20 25 30

Thr Pro Glu Glu Ile Arg Arg Ile Asn Gln Thr Tyr Gln Leu Gln Tyr
35 40 45

Gly Arg Ser Leu Glu Asp Asp Ile Arg Ser Asp Thr Ser Phe Met Phe
50 55 60

Gln Arg Val Leu Val Ser Leu Ser Ala Gly Gly Arg Asp Glu Gly Asn
65 70 75 80

Tyr Leu Asp Asp Ala Leu Val Arg Gln Asp Ala Gln Asp Leu Tyr Glu
85 90 95

Ala Gly Glu Lys Lys Trp Gly Thr Asp Glu Val Lys Phe Leu Thr Val
100 105 110

Leu Cys Ser Arg Asn Arg Asn His Leu Leu His Val Phe Asp Glu Tyr
115 120 125

Lys Arg Ile Ser Gln Lys Asp Ile Glu Gln Ser Ile Lys Ser Glu Thr
130 135 140

Ser Gly Ser Phe Glu Asp Ala Leu Leu Ala Ile Val Lys Cys Met Arg
145 150 155 160

Asn Lys Ser Ala Tyr Phe Ala Glu Lys Leu Tyr Lys Ser Met Lys Gly
165 170 175

Leu Gly Thr Asp Asp Asn Thr Leu Ile Arg Val Met Val Ser Arg Ala
180 185 190

Glu Ile Asp Met Leu Asp Ile Arg Ala His Phe Lys Arg Leu Tyr Gly
195 200 205

Lys Ser Leu Tyr Ser Phe Ile Lys Gly Asp Thr Ser Gly Asp Tyr Arg
210 215 220

Lys Val Leu Leu Val Leu Cys Gly Gly Asp Asp
225 230 235

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Office of Initial Patent Examination -- Scanning Division

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Application deficiencies found during scanning:

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☐ Page(s)_____ of_____ were not present for scanning. (Document title)

☒ ***Scanned copy is best available.*** Drawing figures 9A & 9B are dark